

Gamma Irradiation of Hydrolyzed  
Heart Valve Cusps in the Presence of PPG 400

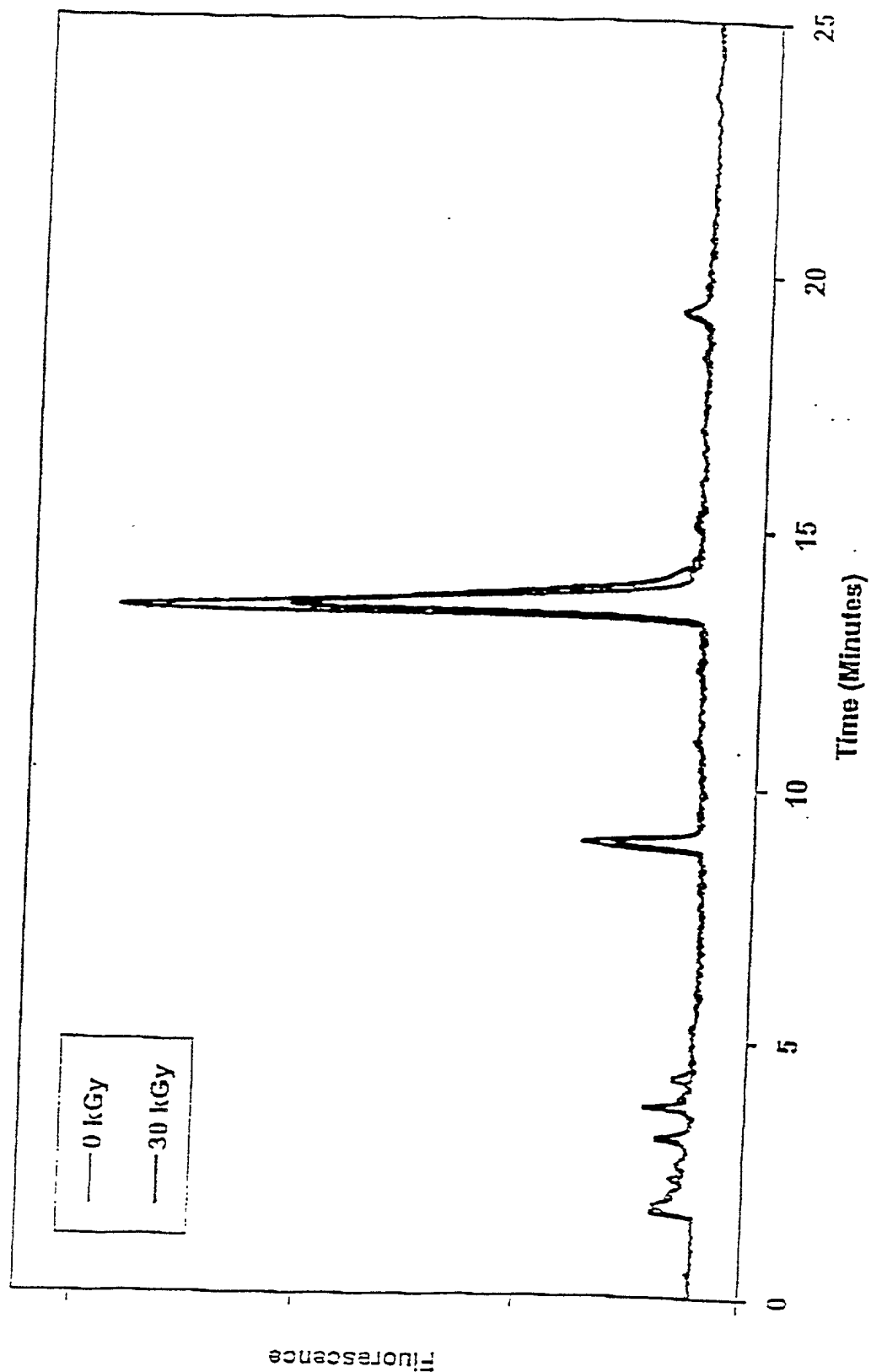


Figure 1a

Gamma Irradiation of Hydrolyzed Heart Valve  
Cusps in the Presence of PPG 400 and 125 mM Trolox C

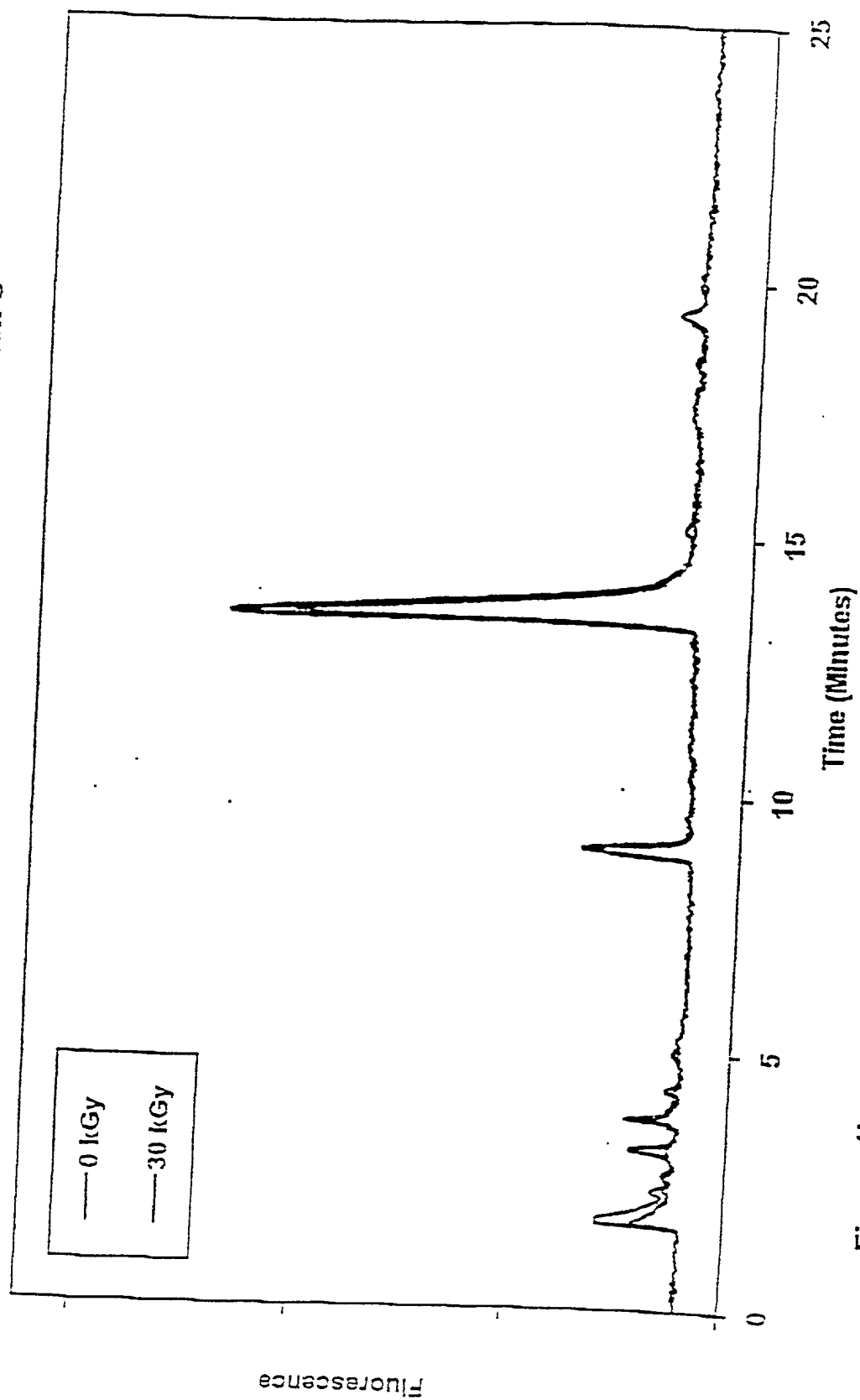


Figure 1b

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence  
of PPG 400 and a Stabilizer Mixture of 62.5mM Trolox, 100mM Lipoic  
Acid, 100mM Coumaric Acid, and 100mM n-Propyl Gallate

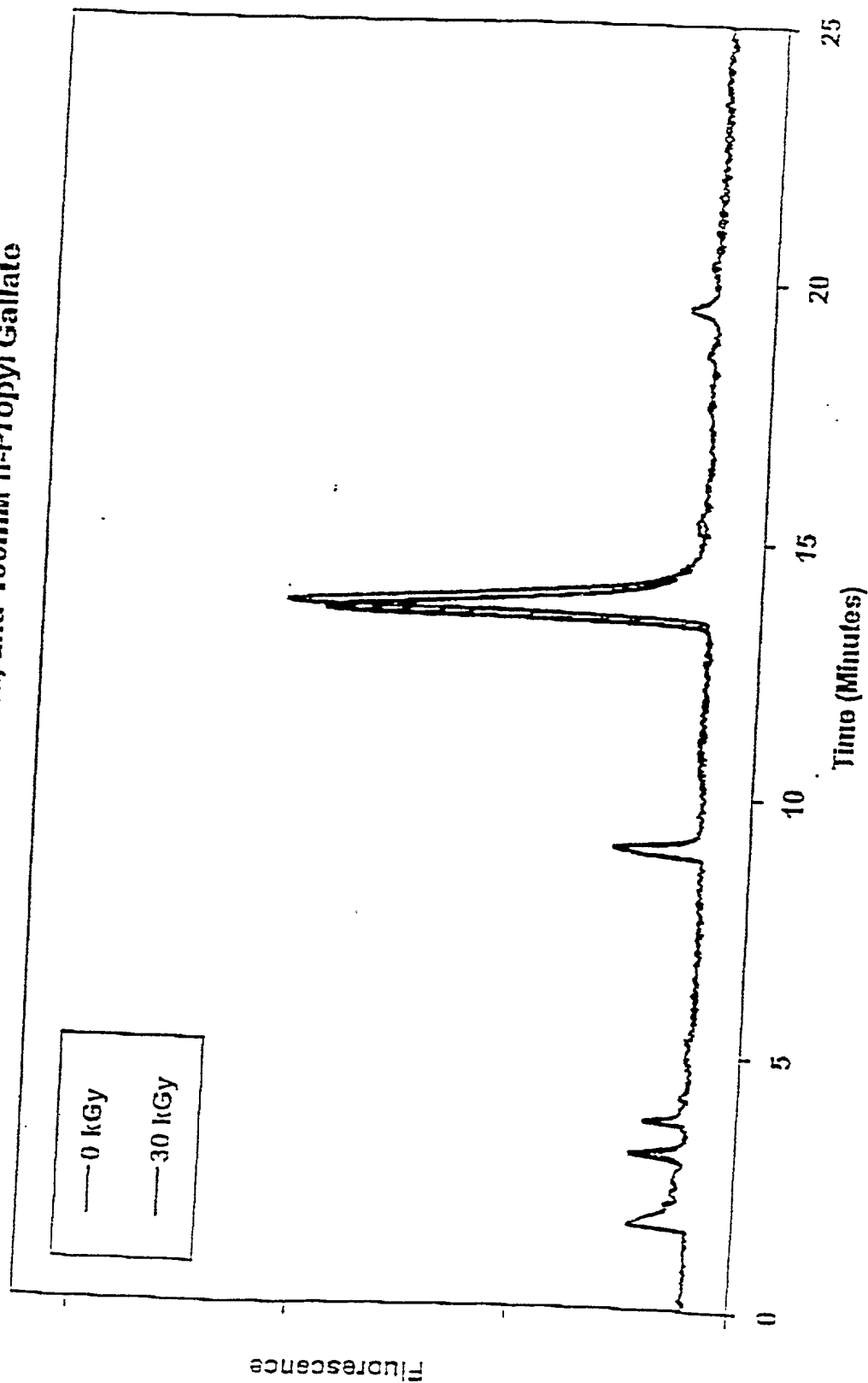


Figure 1c

# Valve Cusps in the Presence of PPG400 with Various Stabilizers

## Gamma Irradiation of Porcine Heart

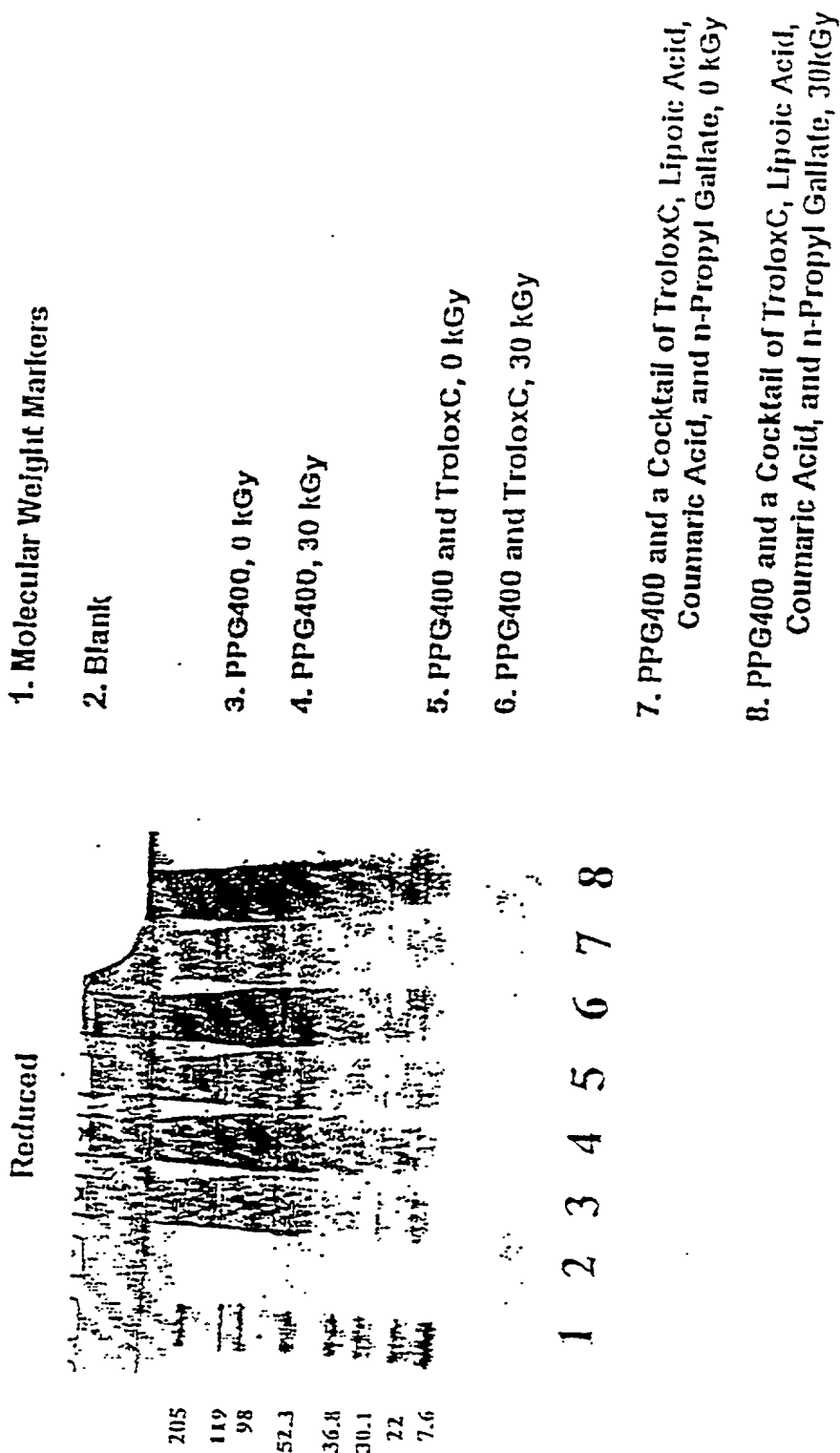


Figure 1d

# Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

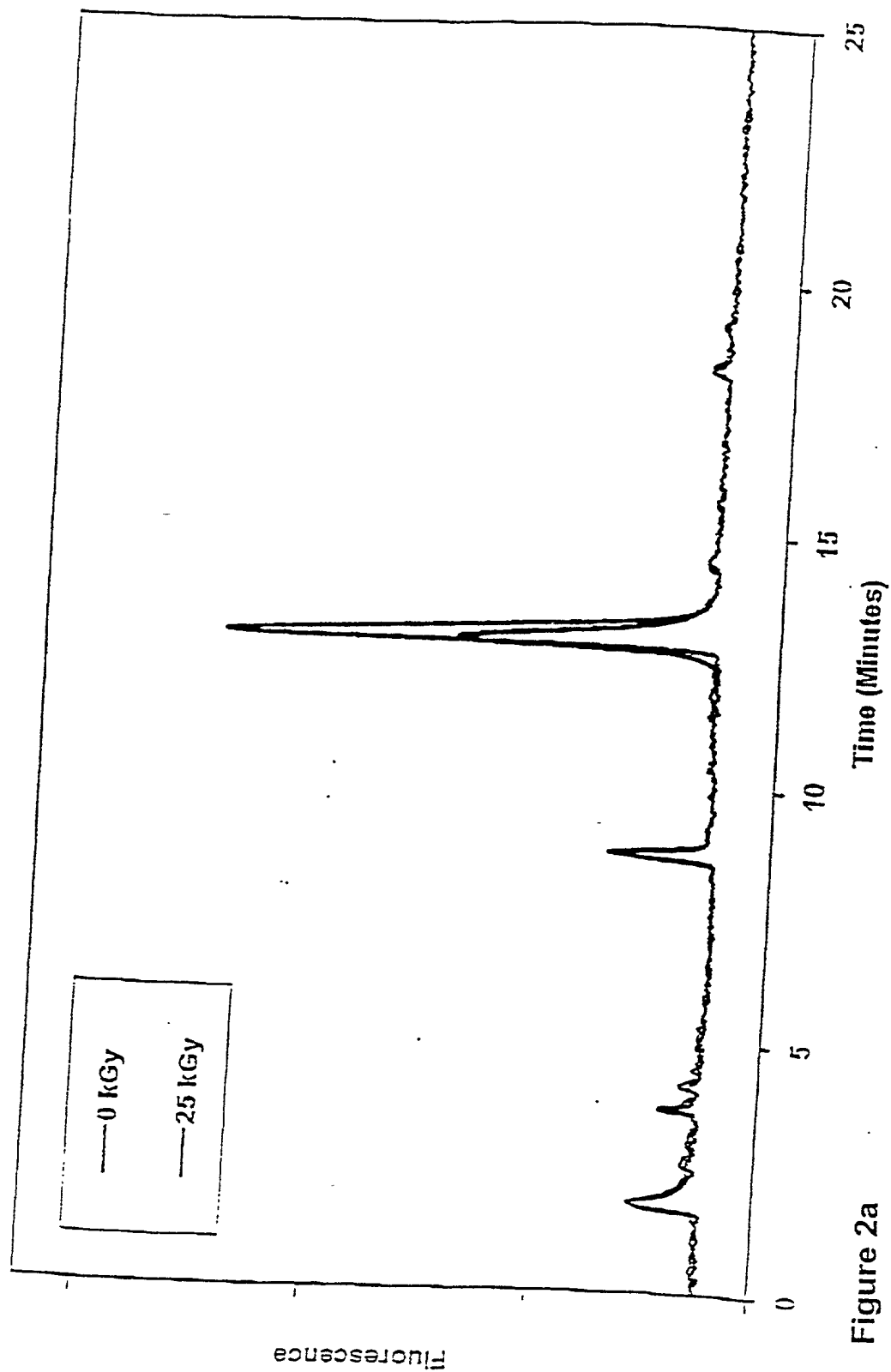
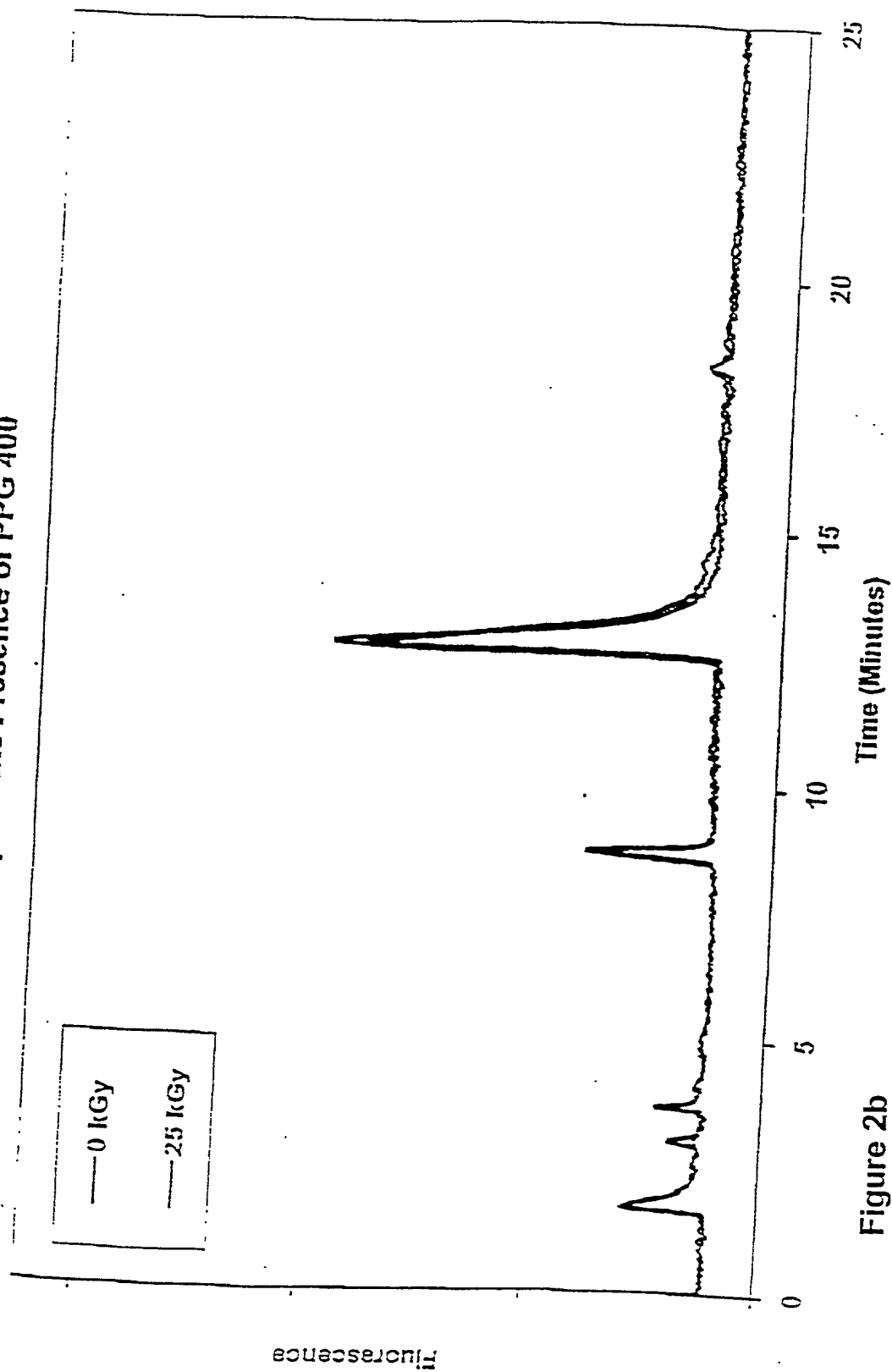


Figure 2a

**Gamma Irradiation of Hydrolyzed Heart Valve  
Cusps in the Presence of PPG 400**



**Figure 2b**

Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
in the Presence of 50% DMSO

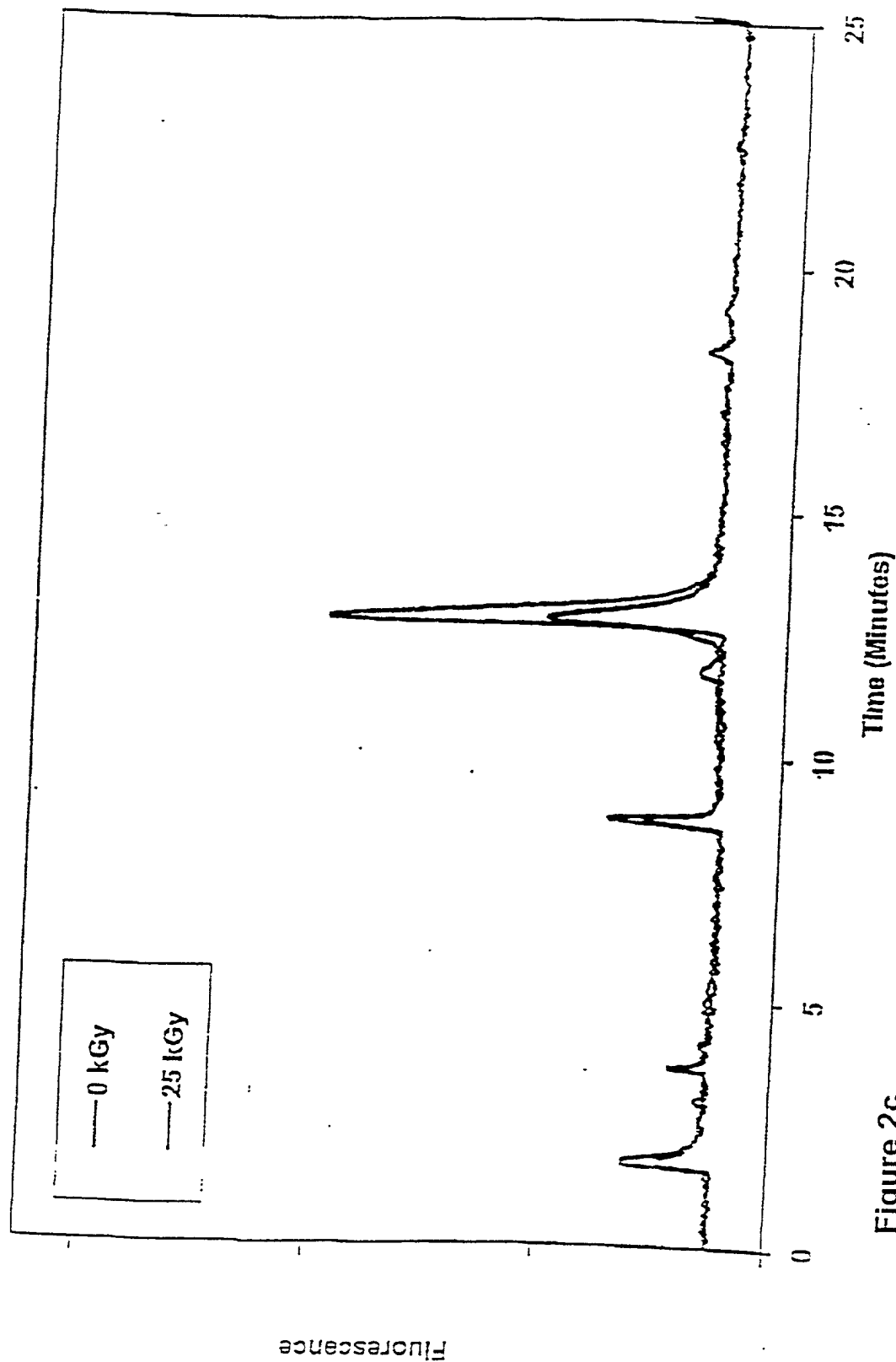
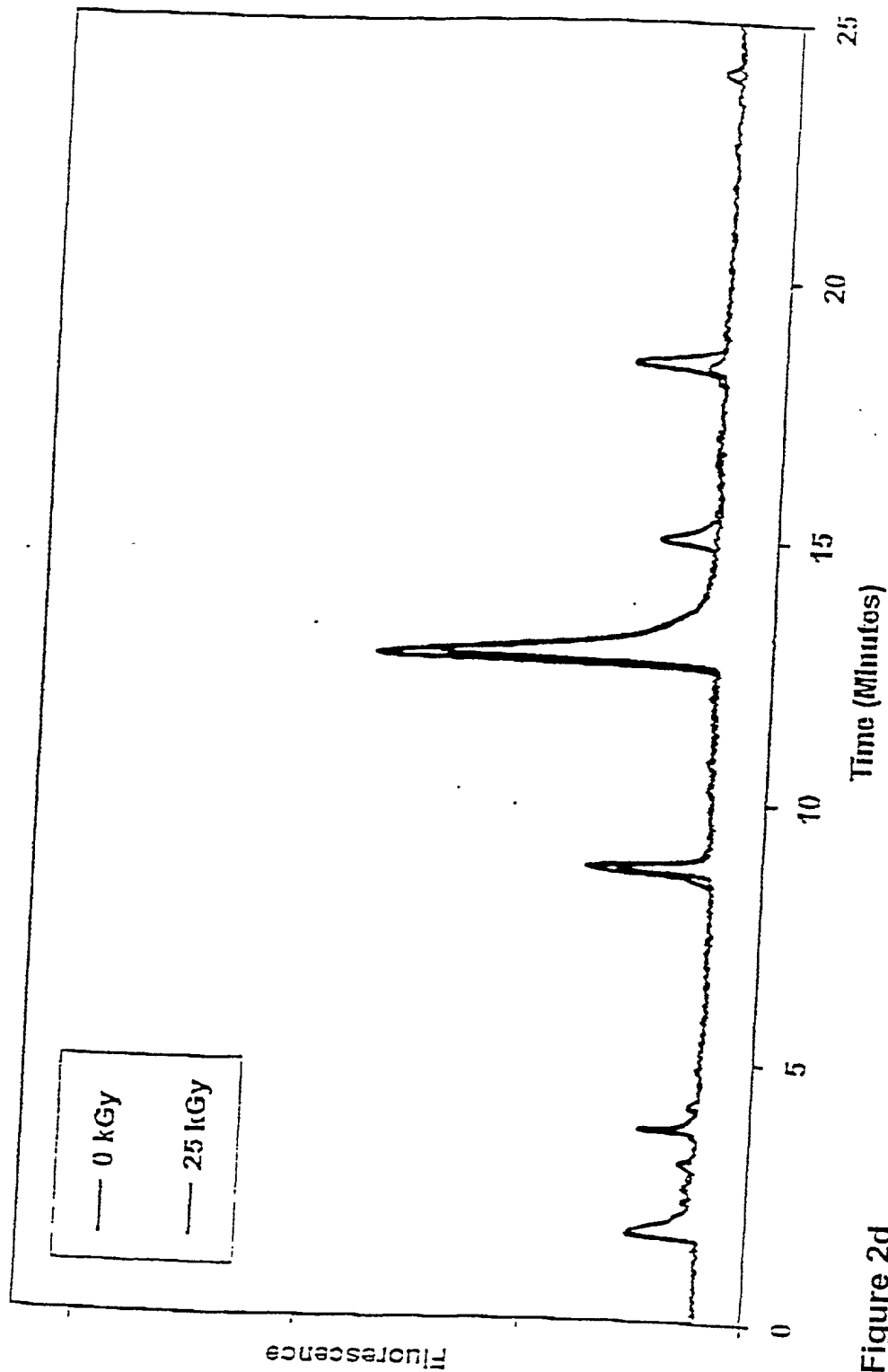


Figure 2c

**Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the  
Presence of 50% DMSO and a Stabilizer Mixture of 167 mM Ascorbate,  
166 mM Coumaric Acid, and 100 mM n-Propyl Gallate**



**Figure 2d**



# Gamma Irradiation of Porcine Heart Valve Cusps in the Presence of Various Solvents

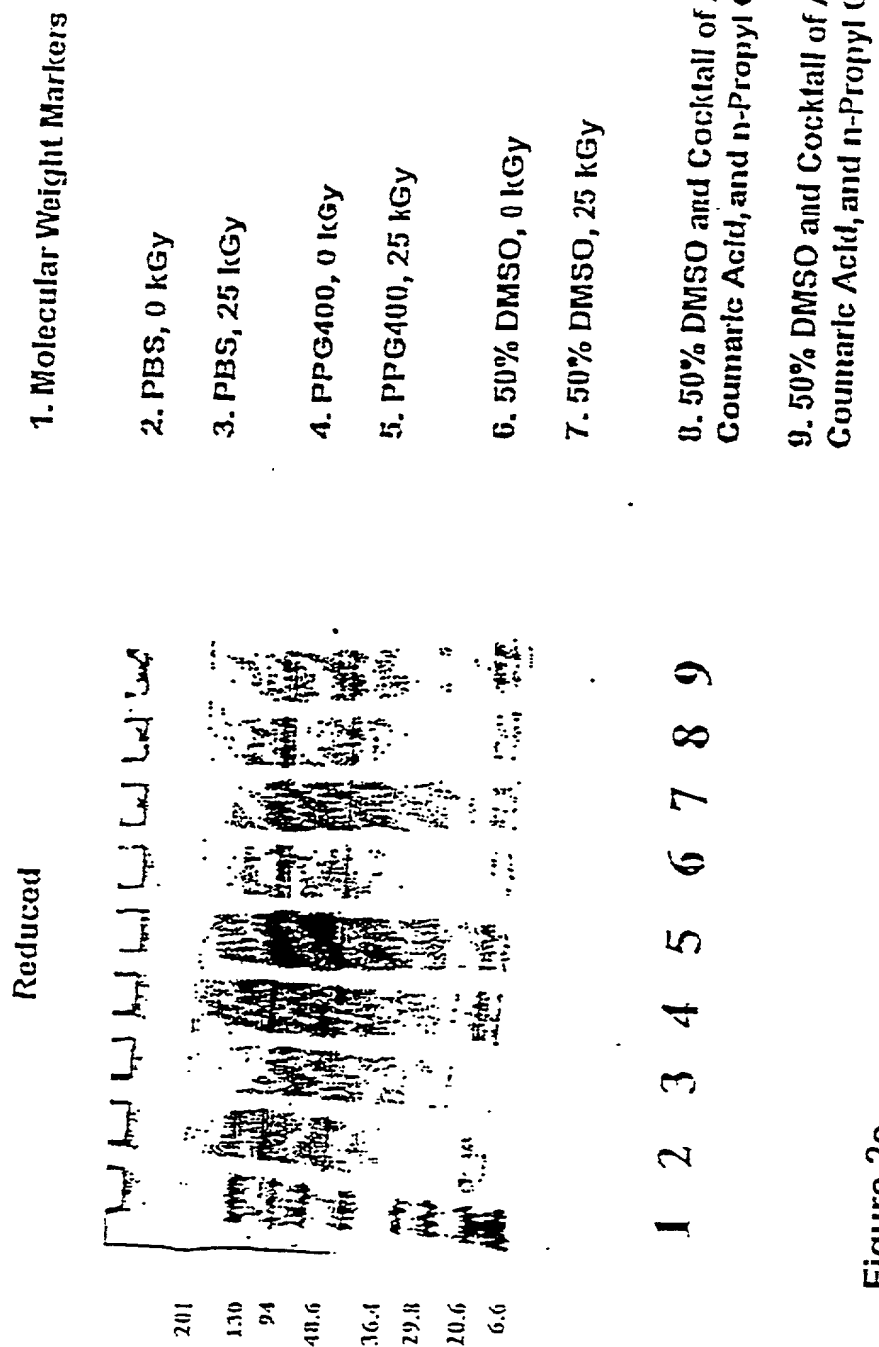


Figure 2e

# Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

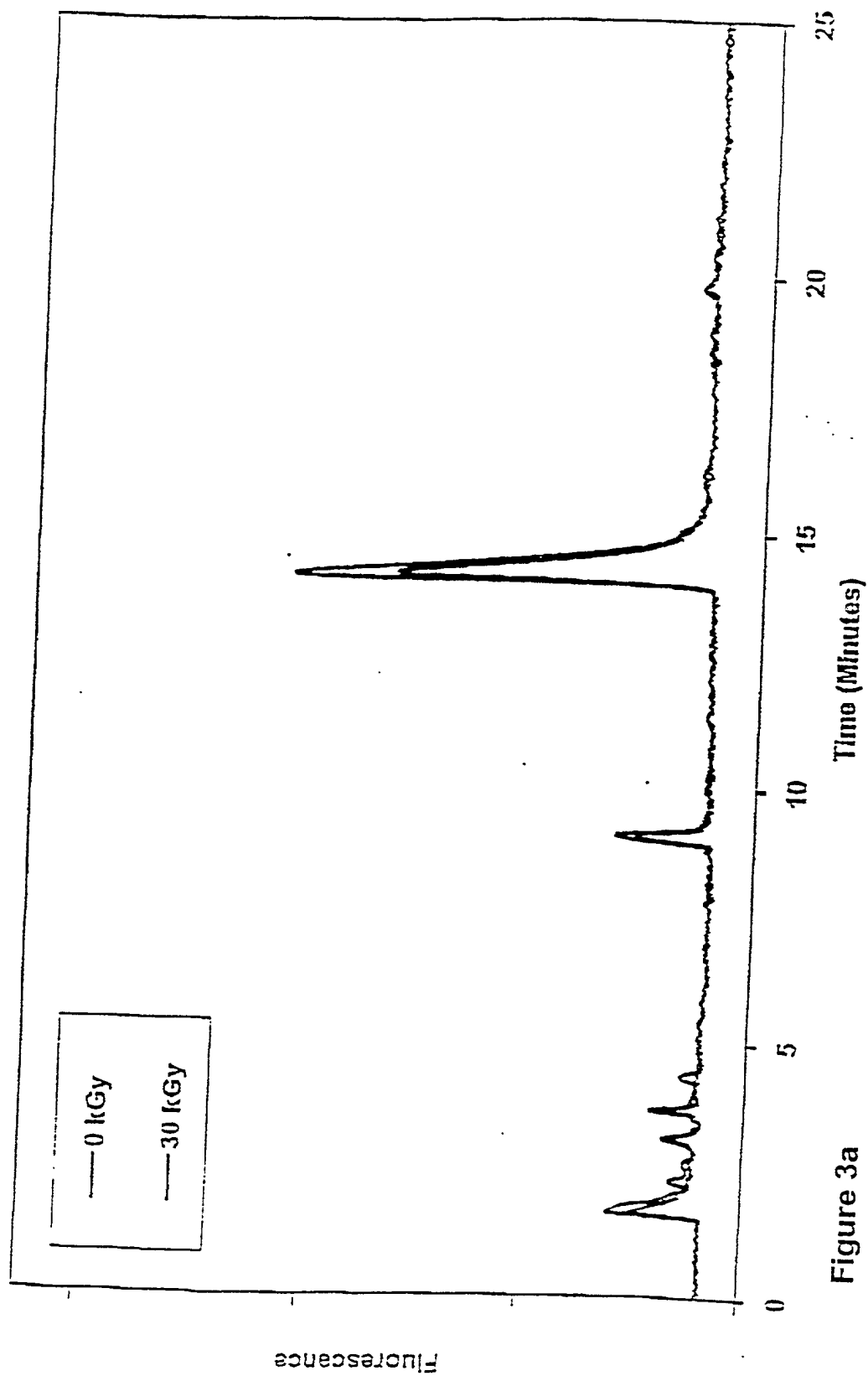


Figure 3a

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the  
Presence of a Cryopreservative (Containing Approximately 20% DMSO)

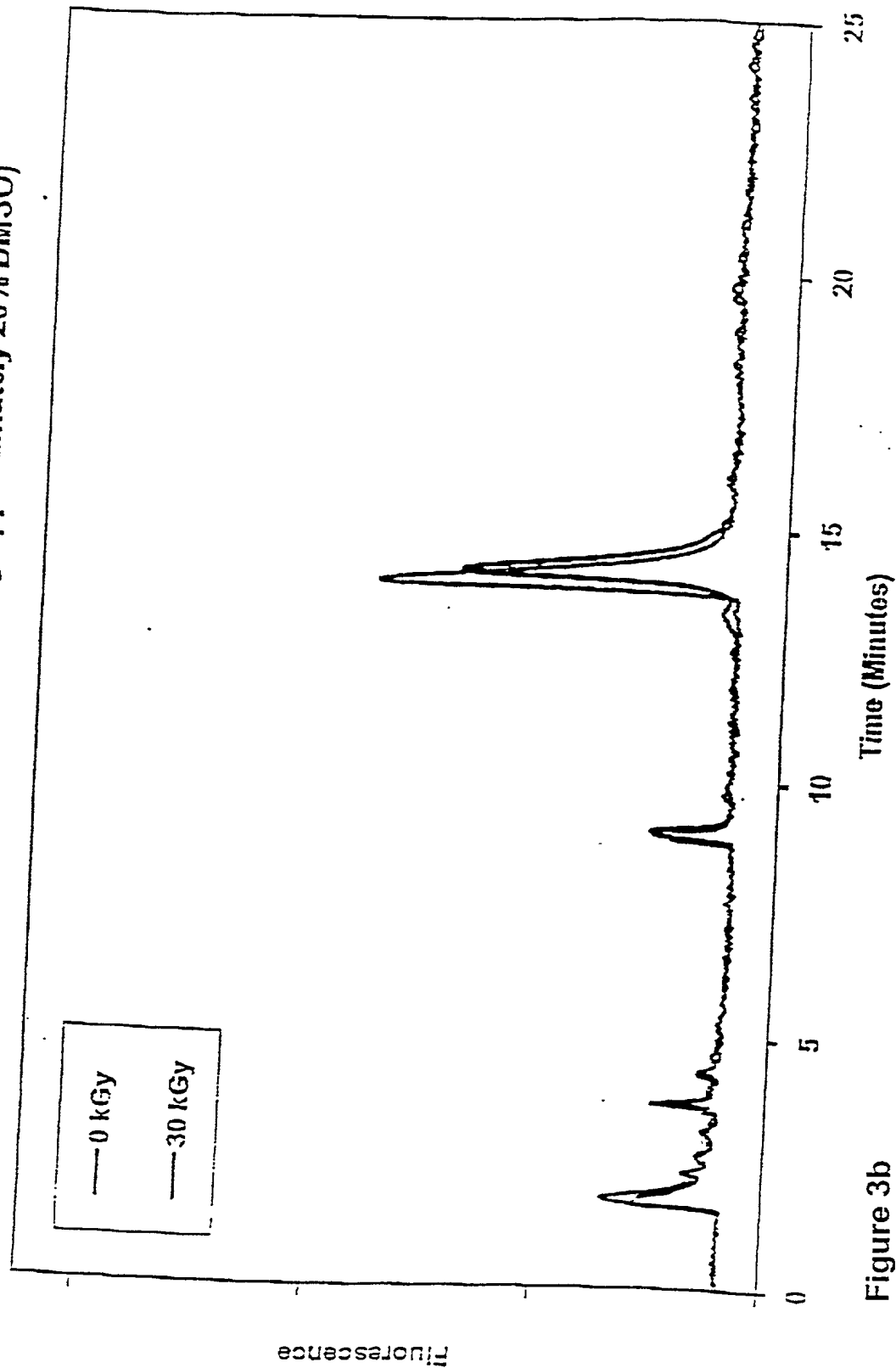
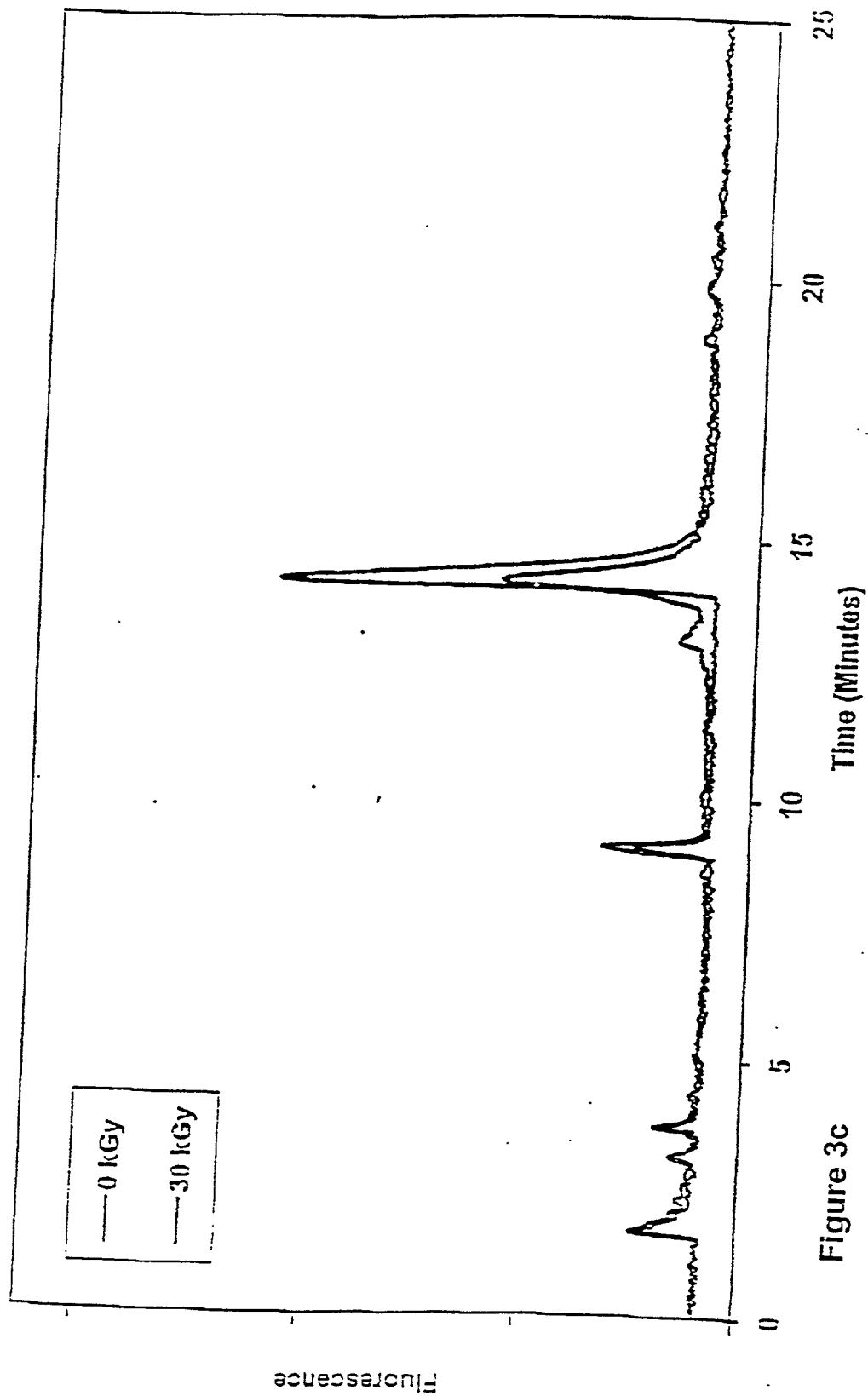


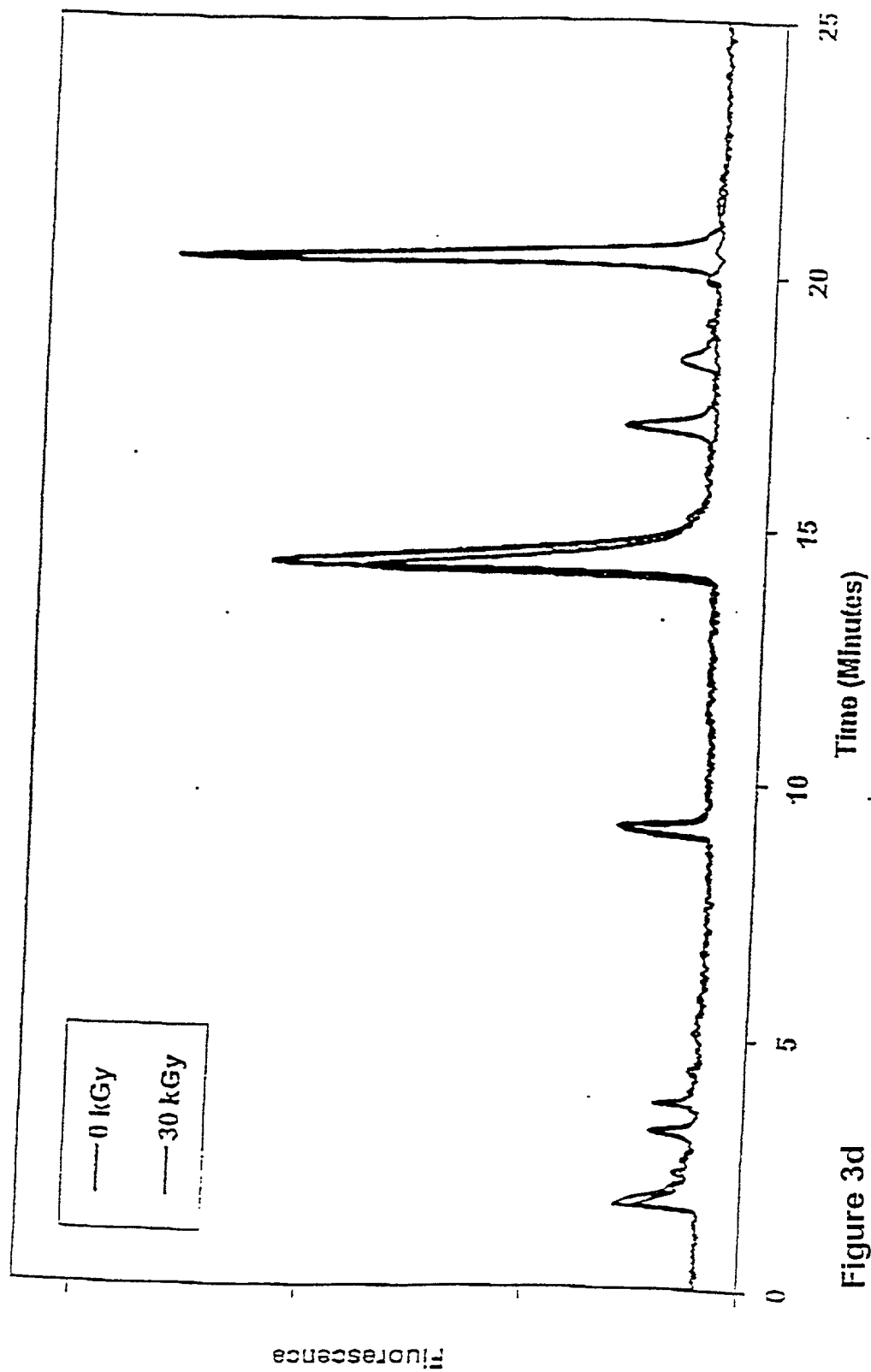
Figure 3b

**Gamma Irradiation of Hydrolyzed Heart Valve  
Cusps in the Presence of 50% DMSO**



**Figure 3c**

**Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the  
Presence of 50% DMSO and Ascorbate**



**Figure 3d**

# Gamma Irradiation of Porcine Heart Valve Cusps in the Presence of Various Solvents

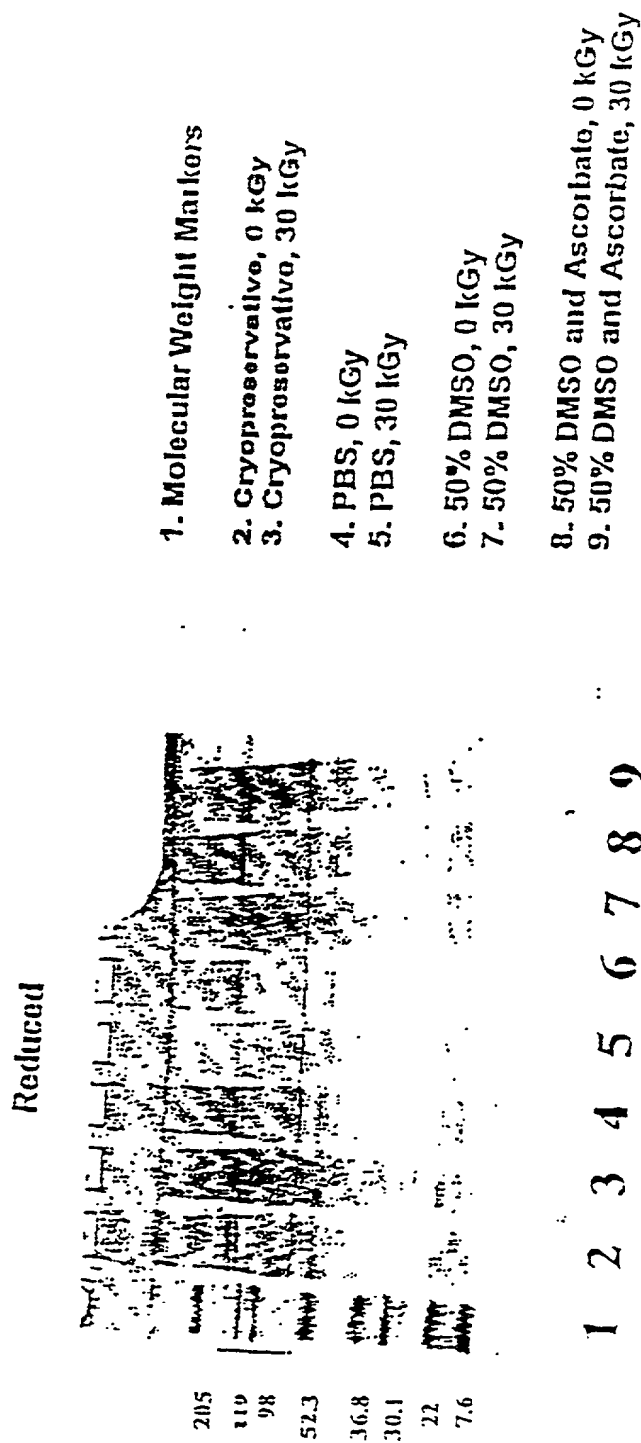


Figure 3e

# Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

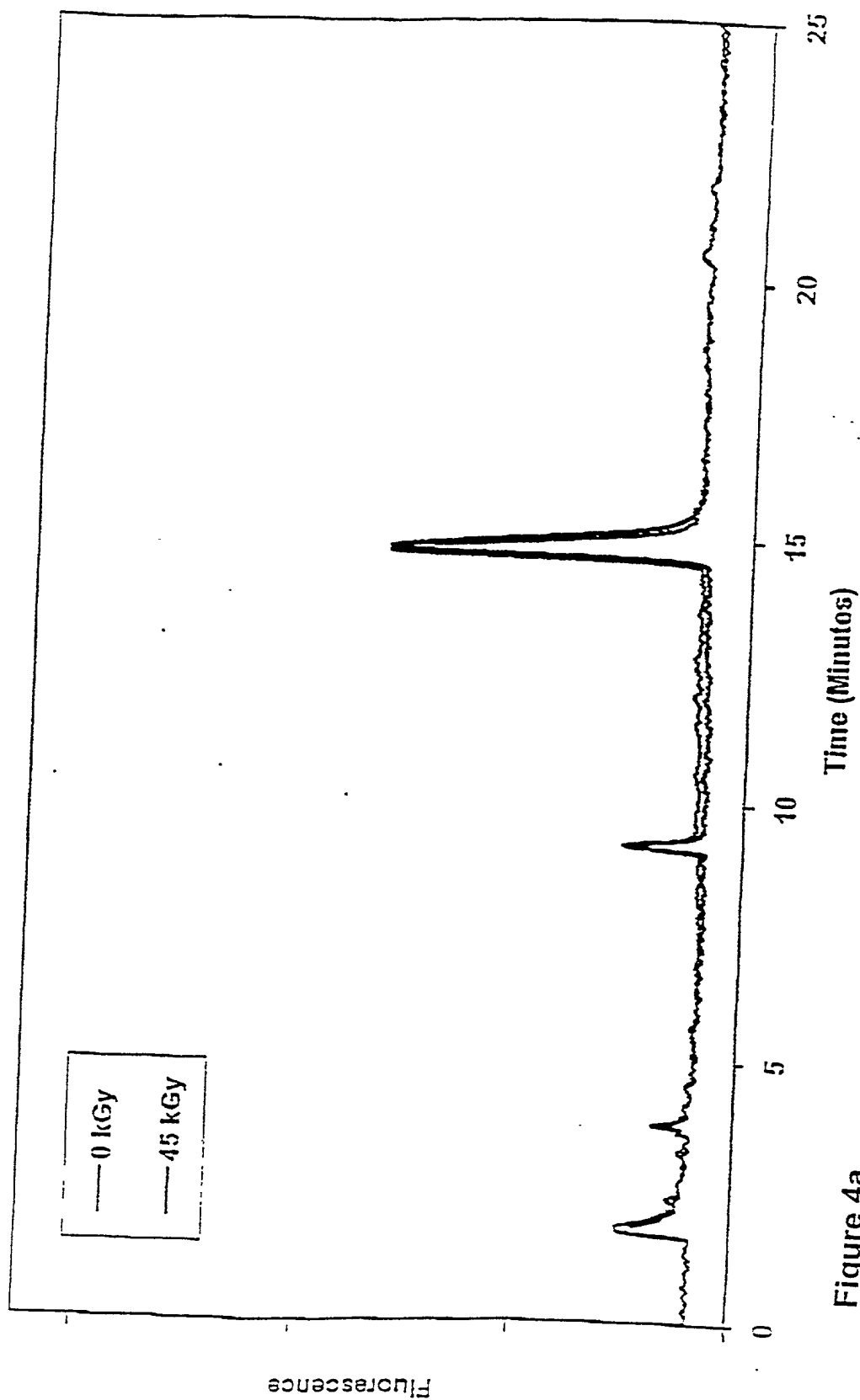
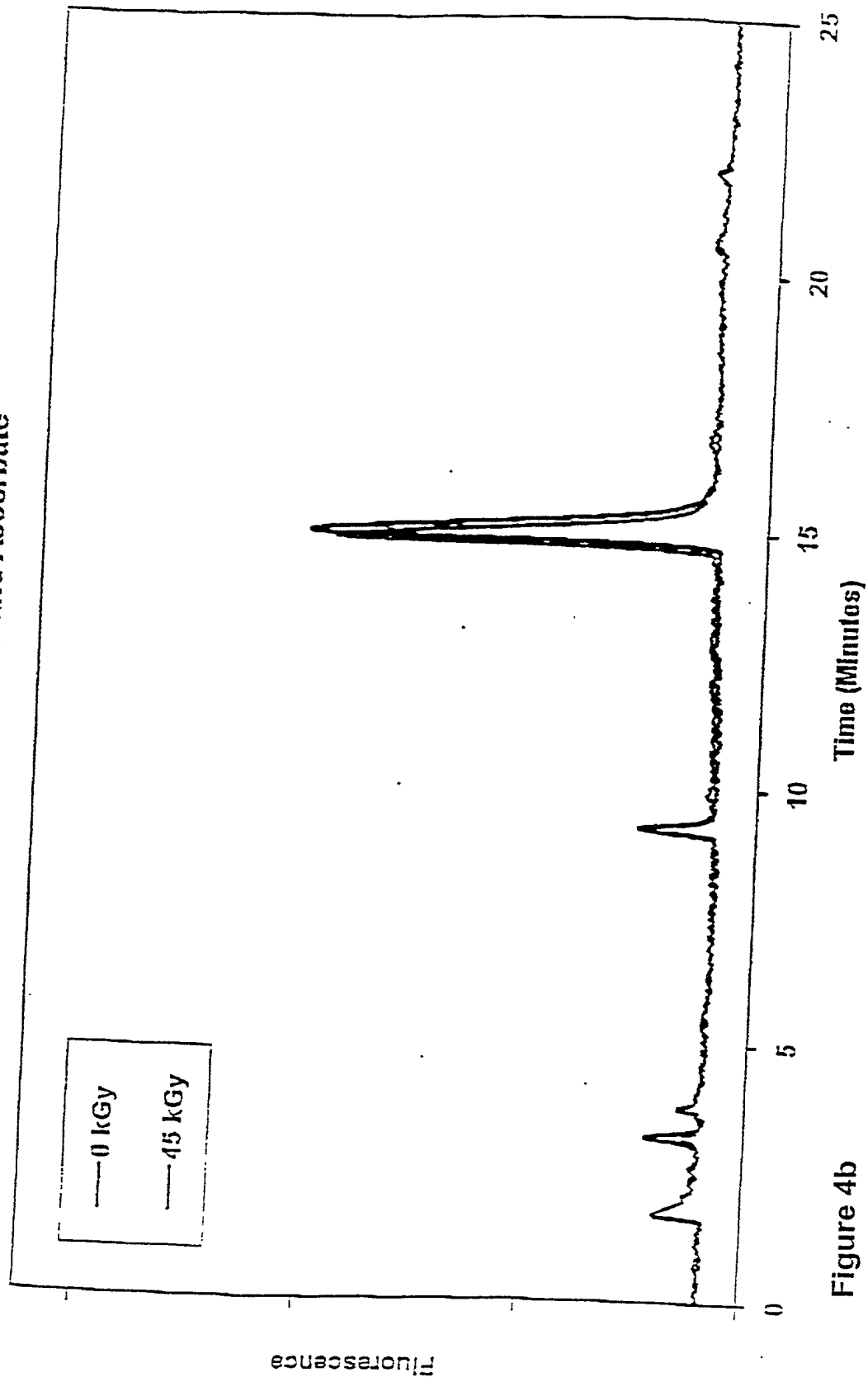


Figure 4a

**Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
in the Presence of PBS and Ascorbate**



**Figure 4b**



Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
in the Presence of PPG 400

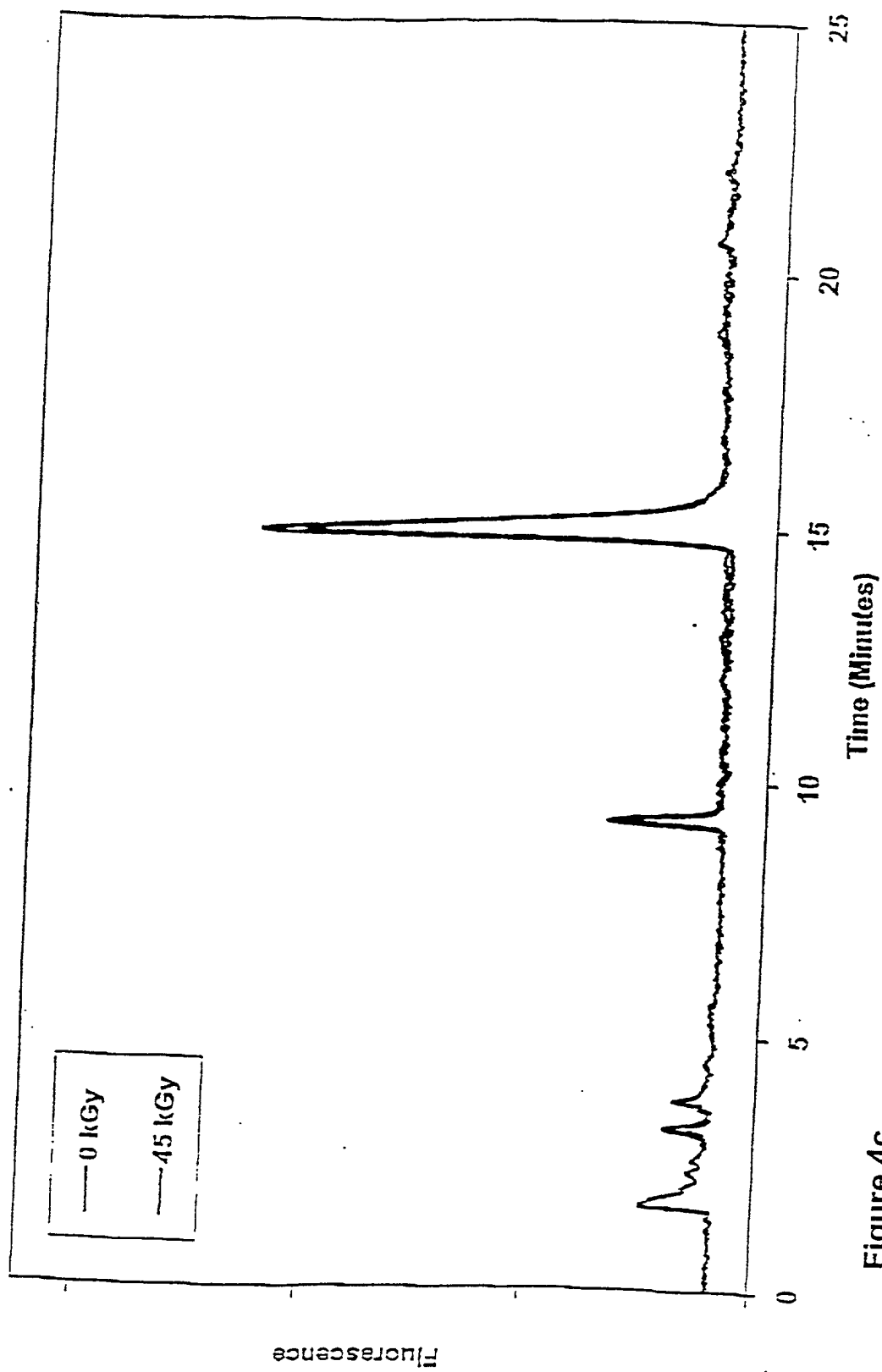


Figure 4c

Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
Dehydrated with PPG 400 and Rehydrated in the Presence of  
PBS and Ascorbate

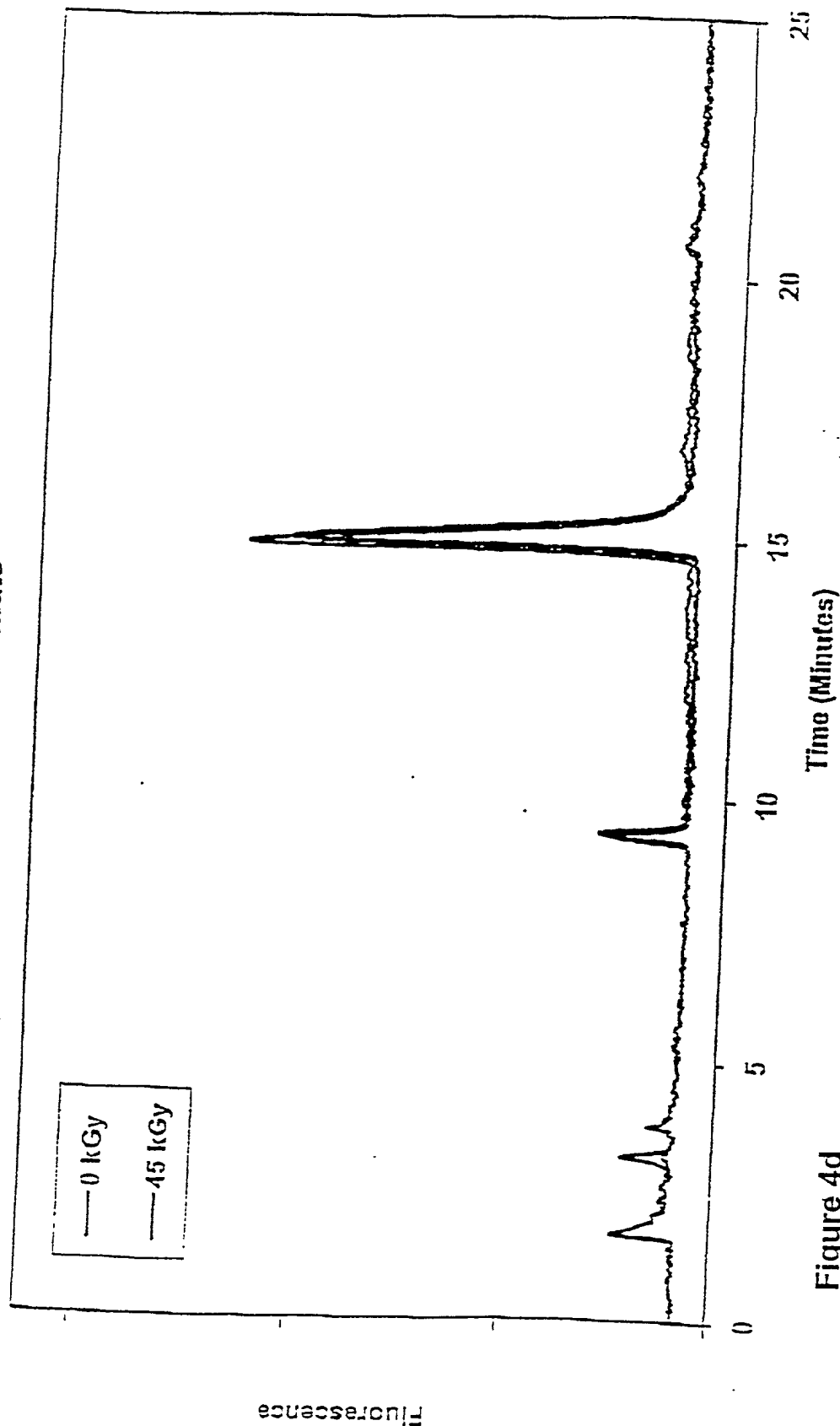


Figure 4d

Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
in the Presence of 50% DMSO

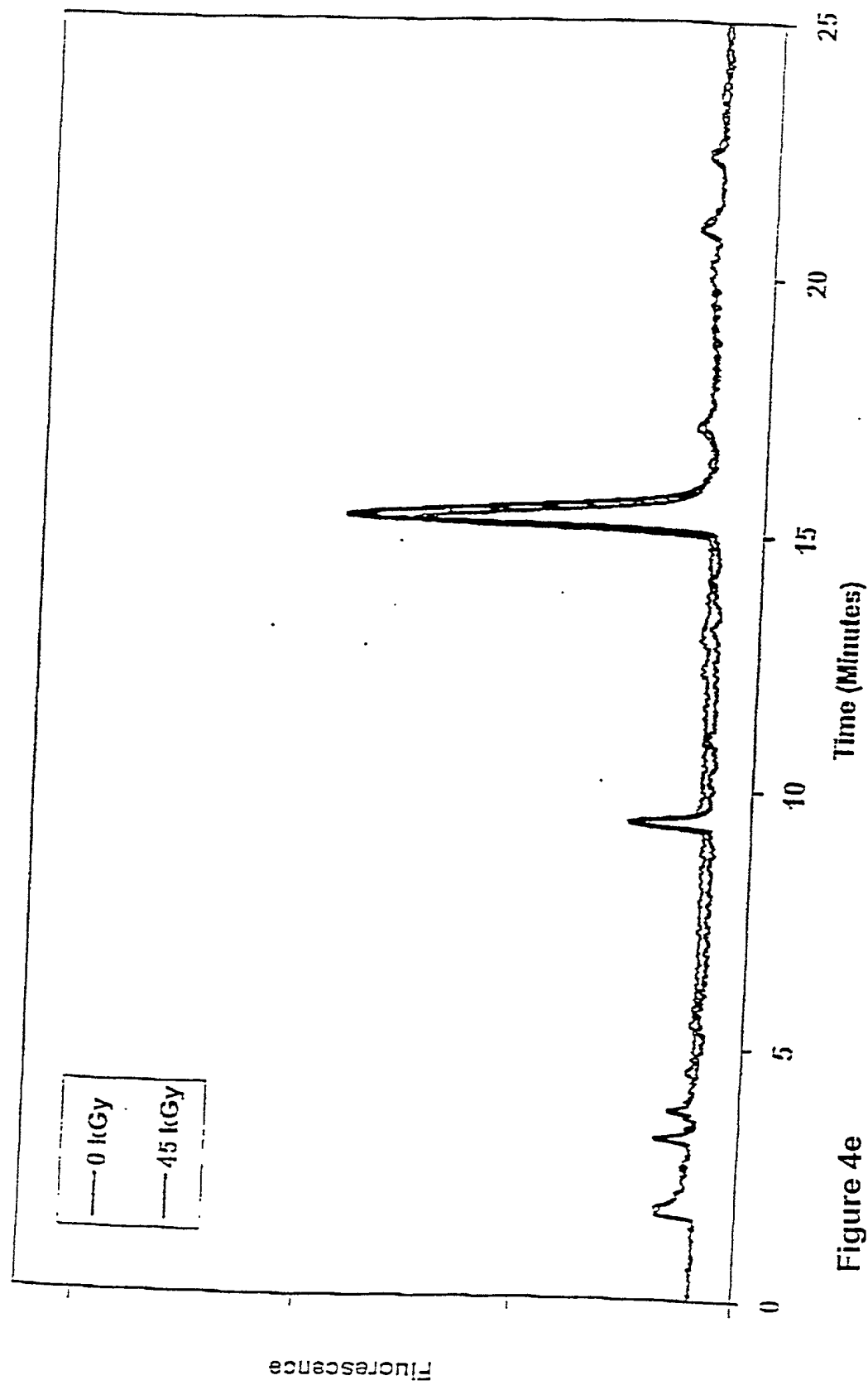
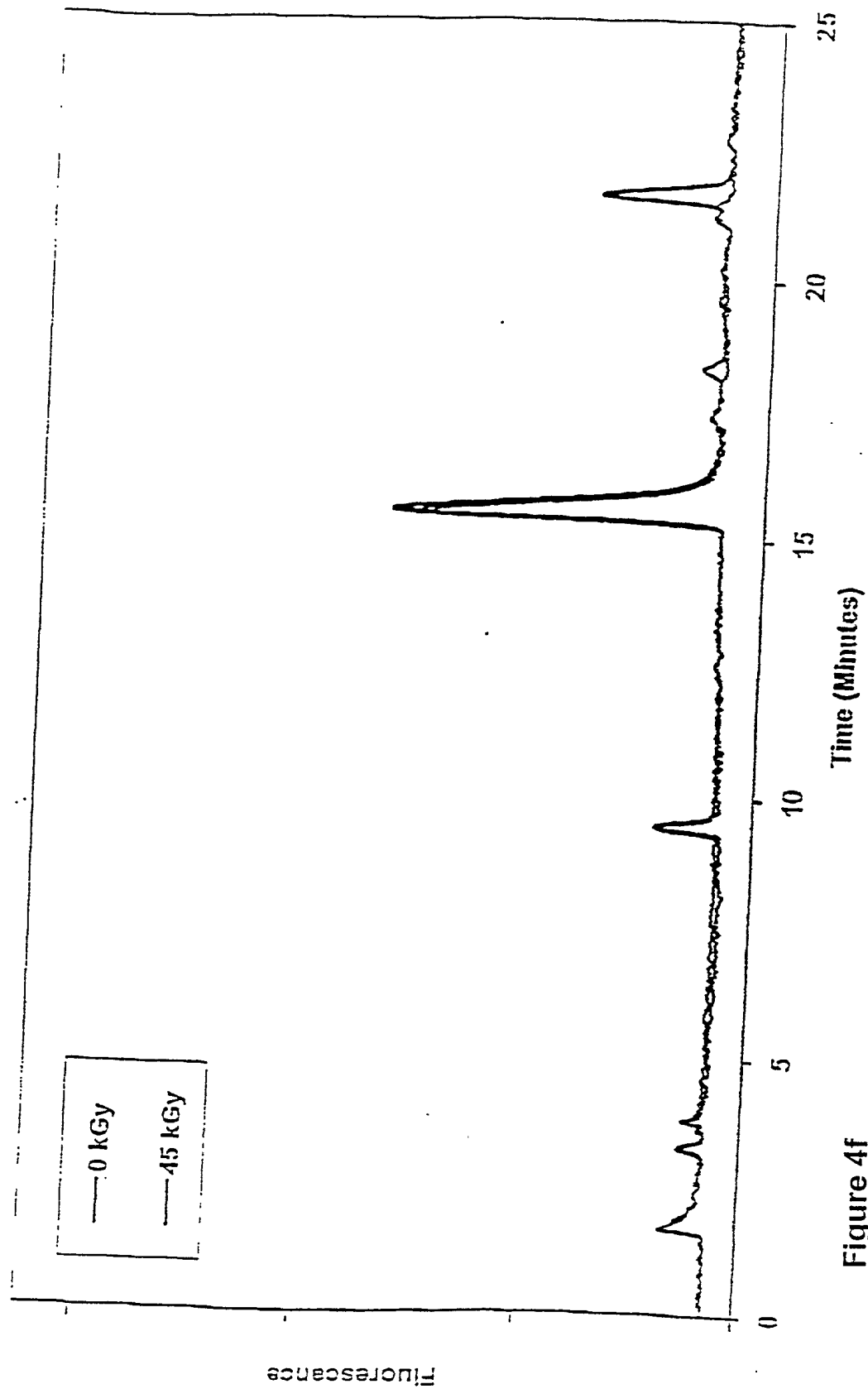


Figure 4e

**Gamma Irradiation of Hydrolyzed Heart Valve Cusps  
in the Presence of 50% DMSO and Ascorbate**



**Figure 4f**

# Gamma Irradiation of Porcine Heart

1. Molecular Weight Markers

2. PBS, 0 kGy

3. PBS, 45 kGy

4. PBS and Ascorbate, 0 kGy

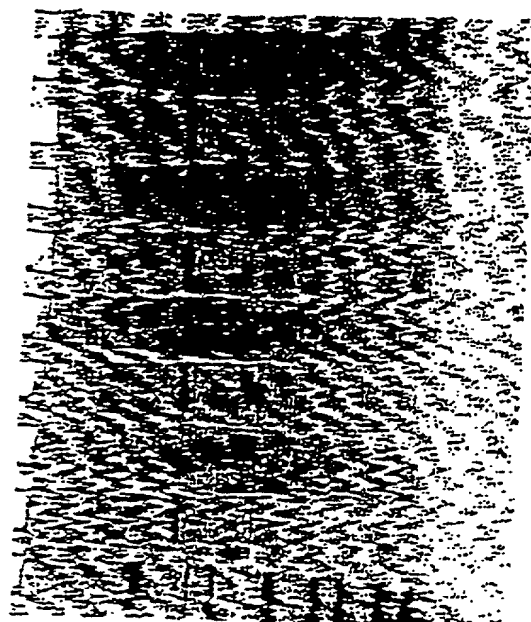
5. PBS and Ascorbate, 45 kGy

6. PPG400, 0 kGy

7. PPG400, 45 kGy

8. Dehydrated in PPG400 and Rehydrated  
with PBS and Ascorbate, 0 kGy

9. Dehydrated in PPG400 and Rehydrated  
with PBS and Ascorbate, 45 kGy



205  
119  
98  
52.3  
36.8  
30.1  
22  
7.6

1 2 3 4 5 6 7 8 9

Figure 4g

# Valve Cusps in the Presence of Various Solvents

## Gamma Irradiation of Porcine Heart

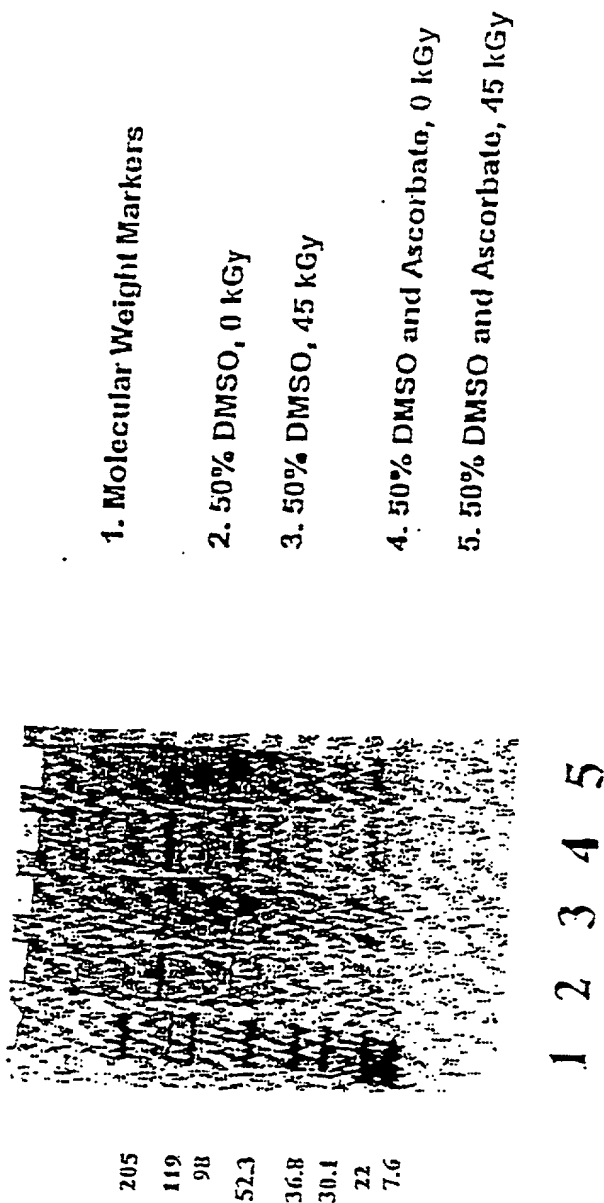
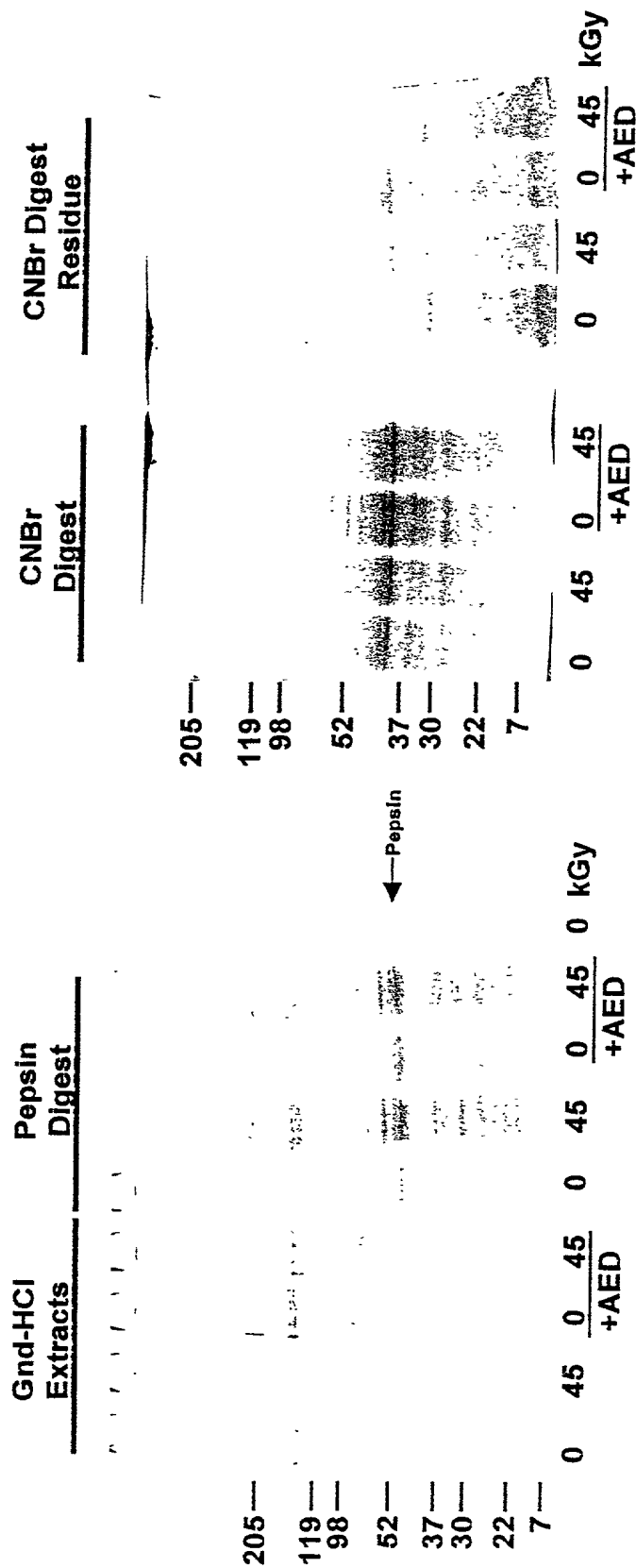


Figure 4h

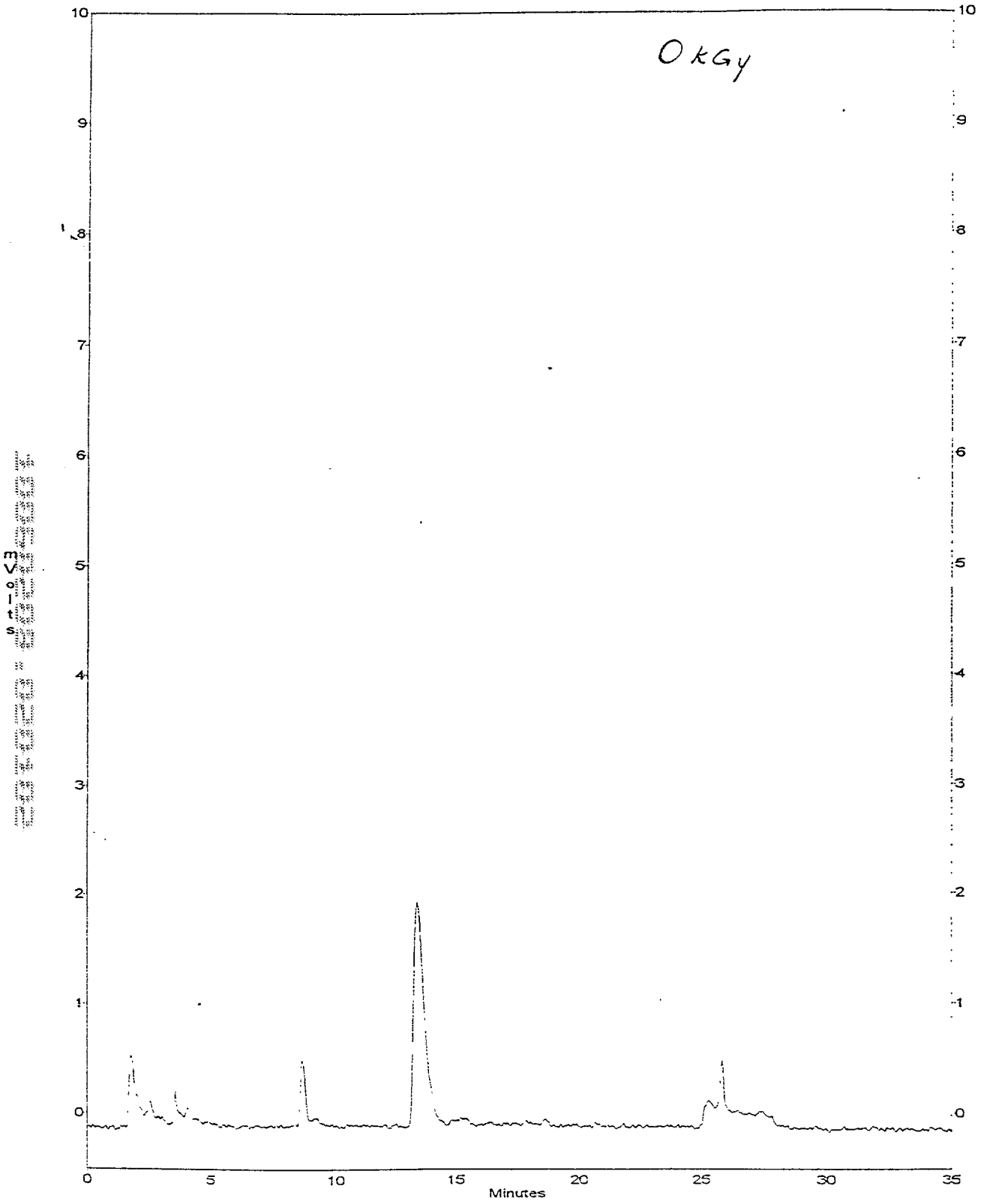
# ACL Gamma Irradiated at -80°C



AED = 100 mM Ascorbate, 22 mM Ergothionine, 100 mM Deferoxamine

SA

c:\class-vp\methods\1028, Channel B

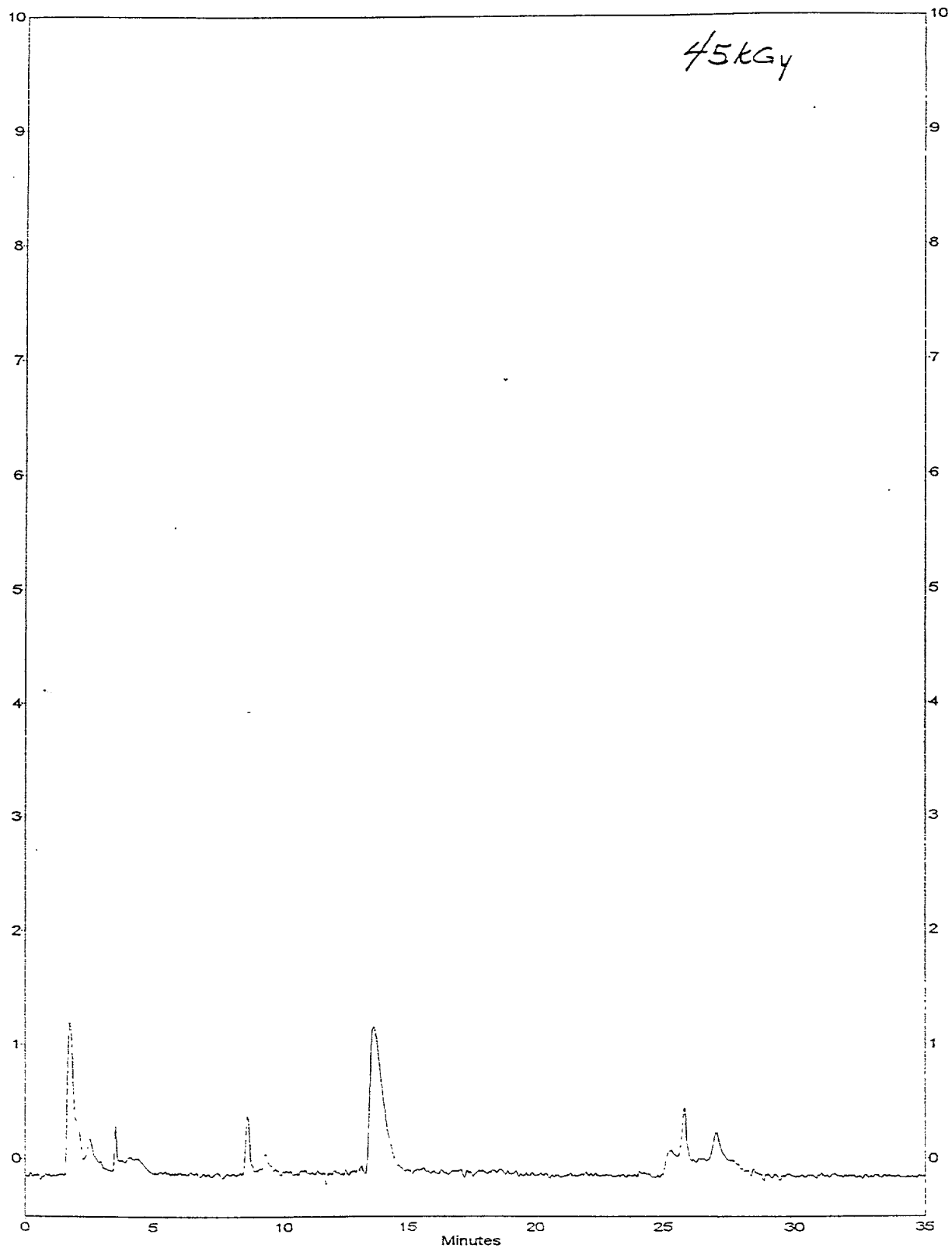


Counts

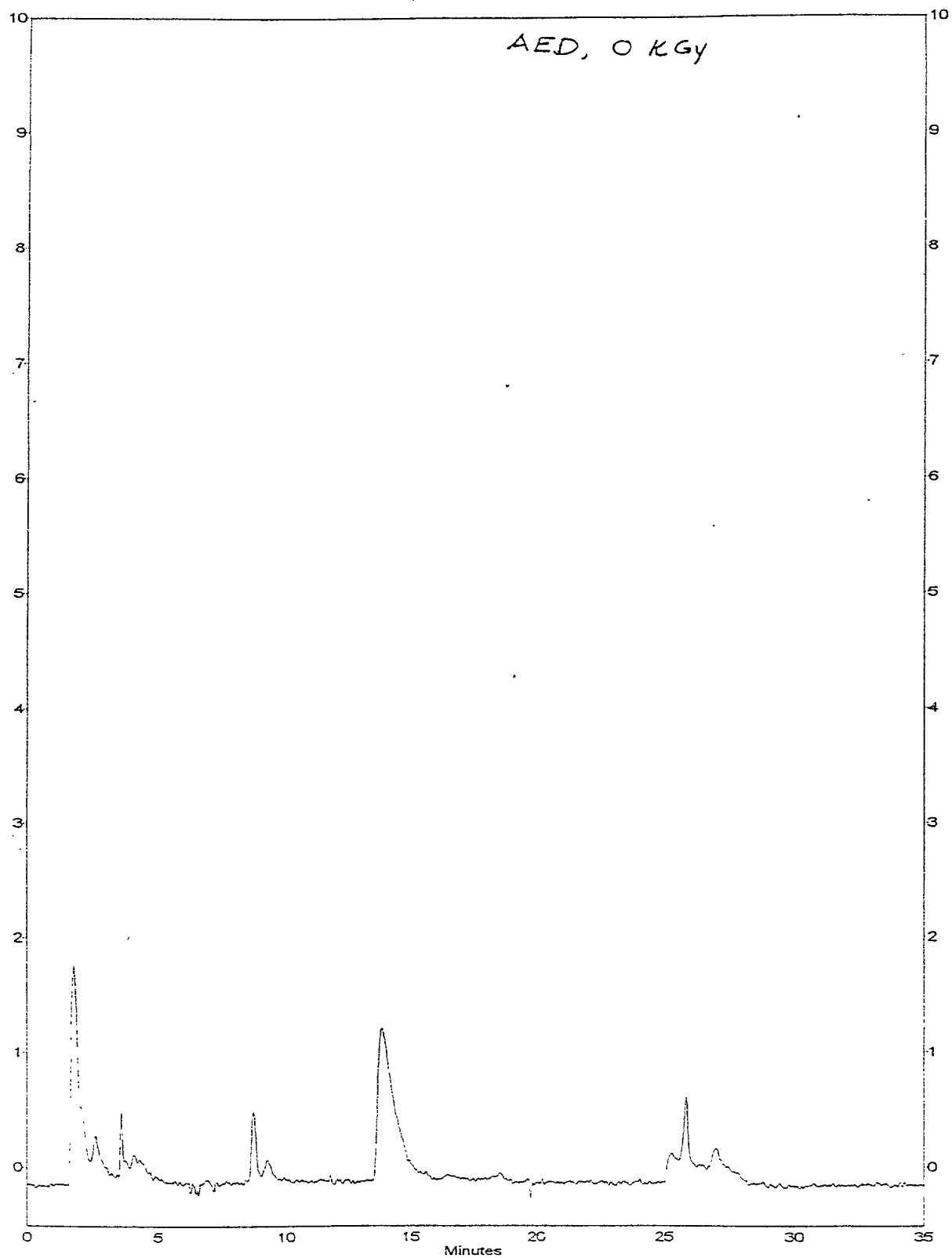
SB



c:\class-vp\methods\1029, Channel B

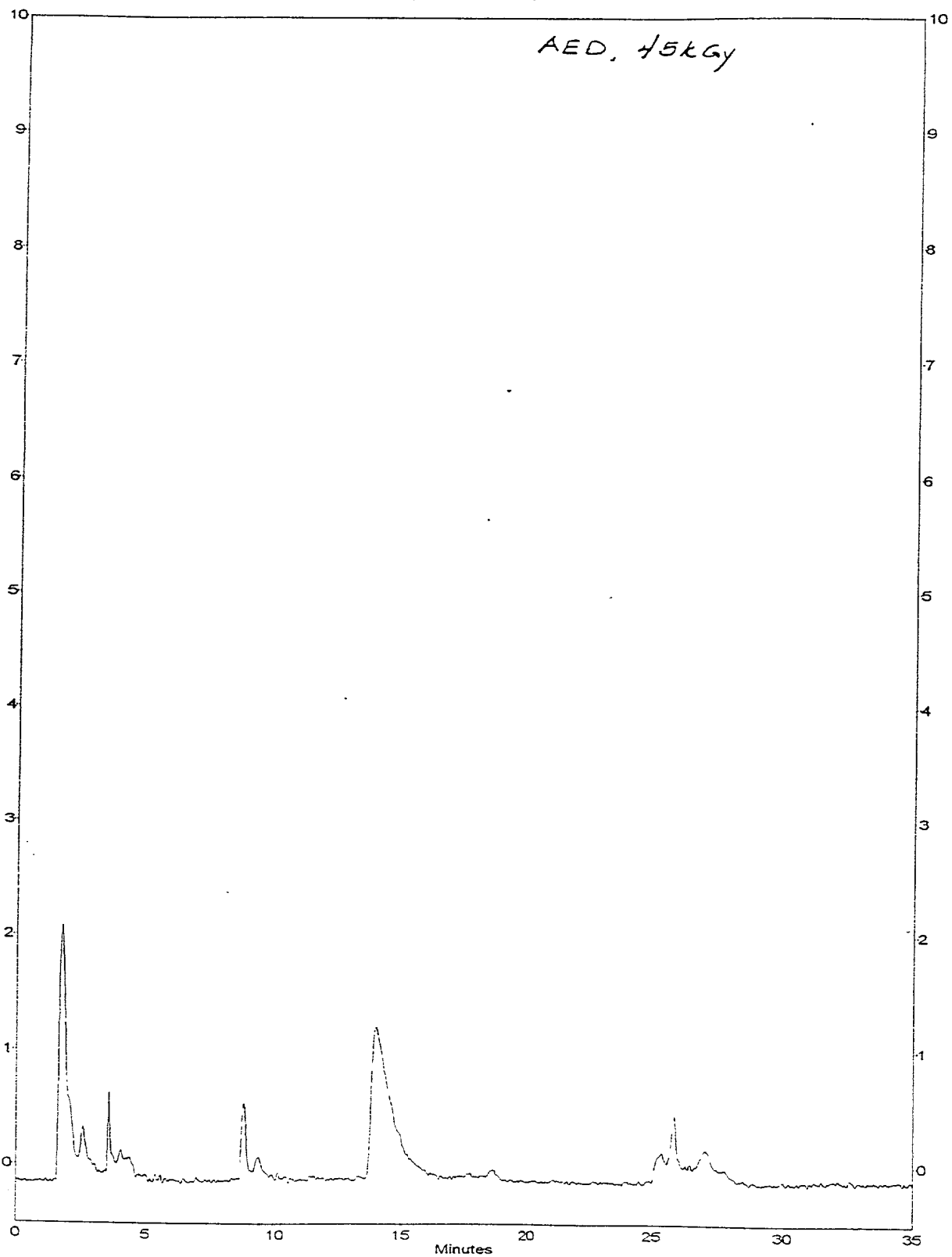


c:\class-vp\methods\1030, Channel B



5D

c:\class-vp\methods\1031, Channel B



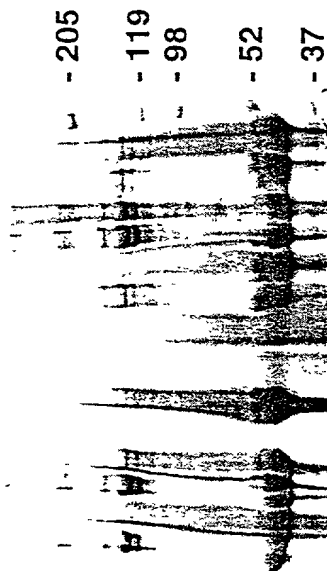
Volts

SE

γ-irradiation of Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C  
1.667kGy/hr

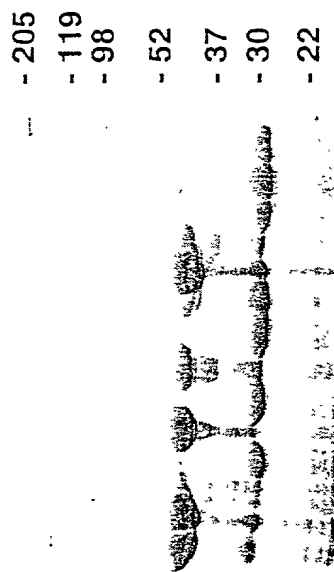
Pepsin Digest

45k	0k	45k	0k	45k	0k	45k	0k	45k	0k	45k	0k
-	Asc	Ergo	His	PolyK	Coctails	Thio					



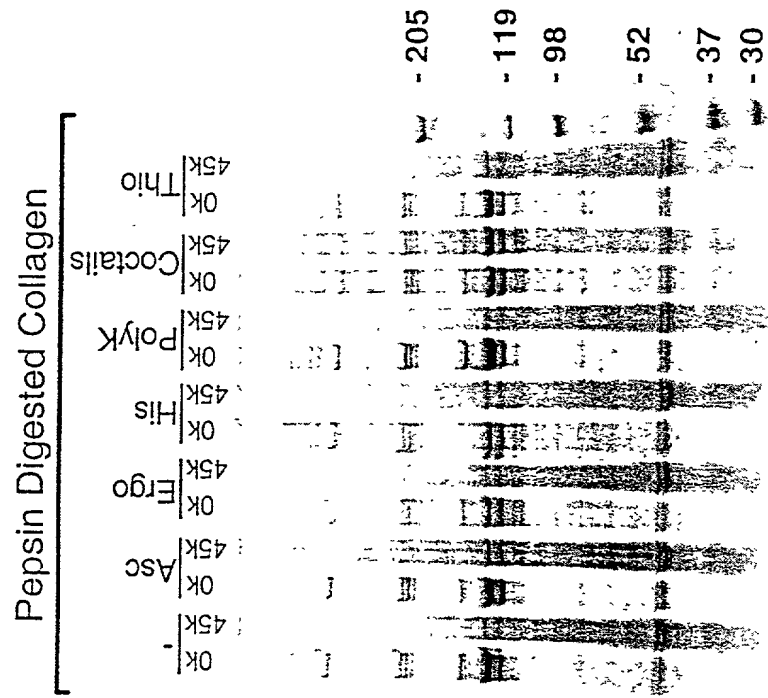
Trypsin Digest

45k	0k	45k	0k	45k	0k	45k	0k	45k	0k	45k	0k
-	Asc	Ergo	His	PolyK	Coctails	Thio					



6A

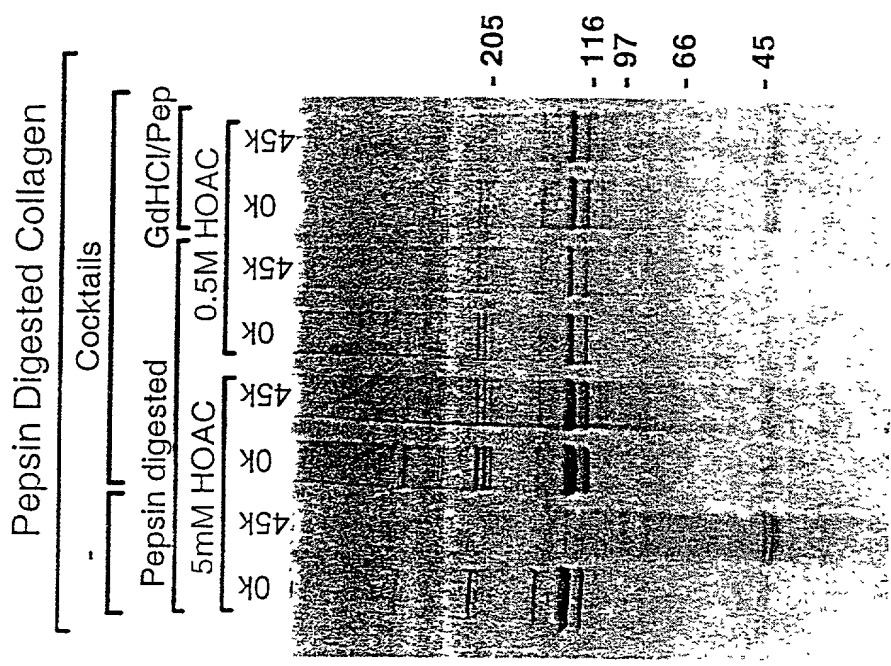
# Pepsin-Digested Collagen Isolated from Irradiated Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C 1.667kGy/hr



Cocktails: PPG/presoak; 100µM trolox, 100mM courmeric acid, 100mM lipoic acid, 100mM n-propyl gallate

CS

Effect of Antioxidants on the Pepsin Digestion of Irradiated Porcine ACL in the Presence of Antioxidants at 4°C 1.667kGy/hr

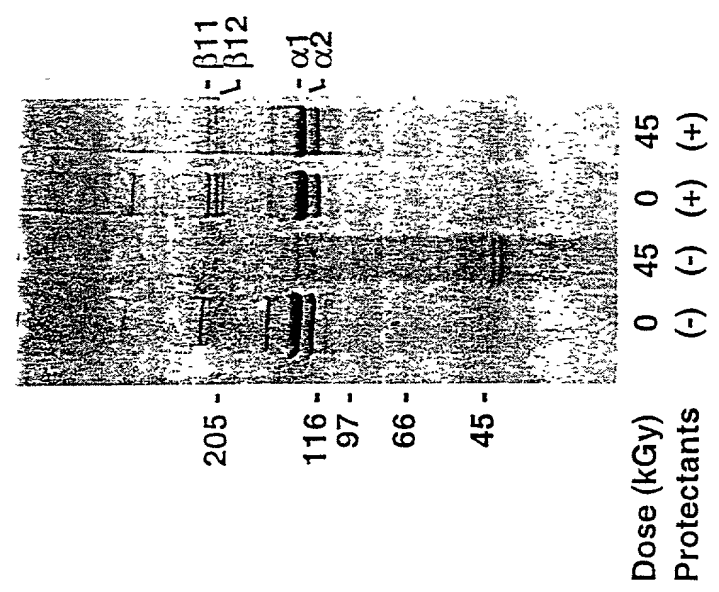


Cocktails: PPG/presoak; 100µM trolox, 100mM ascorbic acid, 100mM lipoic acid, 100mM n-propyl gallate

6C

# Porcine Brainstem Cellulose Isolated from Irradiated Freeze-Dried Porcine ACL in the

presence of various antioxidants at 4°C 1.00 / kGy/hr



Protectants: PPG/presoak;  
 100μM trolox,  
 100mM courmeric acid,  
 100mM lipoic acid, and  
 100mM n-propyl gallate

6D

# Guanidine Extraction of Freeze-dried ACL's

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

205—  
119—  
98—  
52—  
37—  
30—  
22—  
8—

1 = 0 kGy  
2 = 45 kGy  
3 = 200 mM Ascorbate, 0 kGy  
4 = 200 mM Ascorbate, 45 kGy  
5 = 100 mM Thiourea, 0 kGy  
6 = 100 mM Thiourea, 45 kGy  
7 = 200 mM Histidine in PBS, 0 kGy  
8 = 200 mM Histidine in PBS, 45 kGy

9 = 500 mM Trehalose, 0 kGy  
10 = broad range markers  
11 = 500 mM Trehalose, 45 kGy  
12 = 5 mg/ml Ergothionine, 0 kGy  
13 = 5 mg/ml Ergothionine, 45 kGy  
14 = 10 mM Poly-lysine, 0 kGy  
15 = 10 mM Poly-lysine, 45 kGy  
16 = PPG, then cocktail (100µM trolox, 100mM  
courmeric acid, 100mM lipoic acid, 100mM  
propyl gallate), 0 kGy  
17 = PPG, then cocktail, 45 kGy

66



# Purified GAG/Proteoglycans from Irradiated ACL



205 —  
119 —  
98 —  
52 —  
37 —  
30 —  
22 —

## Lane:

- 1 = Recombinant Human Decorin
- 2 = No Scavengers, 0 kGy
- 3 = No Scavengers, 45 kGy
- 4 = 200 mM Ascorbate, 0 kGy
- 5 = 200 mM Ascorbate, 45 kGy
- 6 = 100 mM Thiourea, 0 kGy
- 7 = 100 mM Thiourea, 45 kGy
- 8 = 200 mM Histidine in PBS, 0 kGy
- 9 = 200 mM Histidine in PBS, 45 kGy

## Lane:

- 1 = 500 mM Trehalose, 0 kGy
- 2 = 500 mM Trehalose, 45 kGy
- 3 = 5 mg/ml Ergothionine, 0 kGy
- 4 = 5 mg/ml Ergothionine, 45 kGy
- 5 = 10 mM Poly-lysine, 0 kGy
- 6 = 10 mM Poly-lysine, 45 kGy
- 7 = PPG Pretreatment, then cocktail, 0 kGy
- 8 = PPG Pretreatment, then cocktail, 45 kGy
- 9 = Recombinant Human Decorin

6F

# DEAE Chromatography of Porcine ACL Irradiated in Cryopreservative ± Ascorbate at -80°C at 5.1 kGy/h

## Edmonton Cryopreservative

FC Regulated Quick

## Modified VS55

Regulated Quick

205 —  
119 —  
98 —  
52 —  
37 —  
30 —  
22 —  
8 —

0 0 50 0 50 0 50 0 50  
- - - + + - - + +

0 50 0 50 0 50 0 50  
- - + + - - + +

Edmonton CP: M199, 10% FCS, 2 M DMSO, Pen-Strep  
Modified VS55: 100 mM trehalose, 15 mM KH<sub>2</sub>PO<sub>4</sub>, 42 mM K<sub>2</sub>HPO<sub>4</sub>, 15 mM KCl, 10 mM NaHCO<sub>3</sub>, 150 mM mannitol, 24.2% DMSO,  
16.8% 1,2-propanediol, 14% formamide

FC: fresh ACL control

Quick Freeze: dry-ice ethanol bath

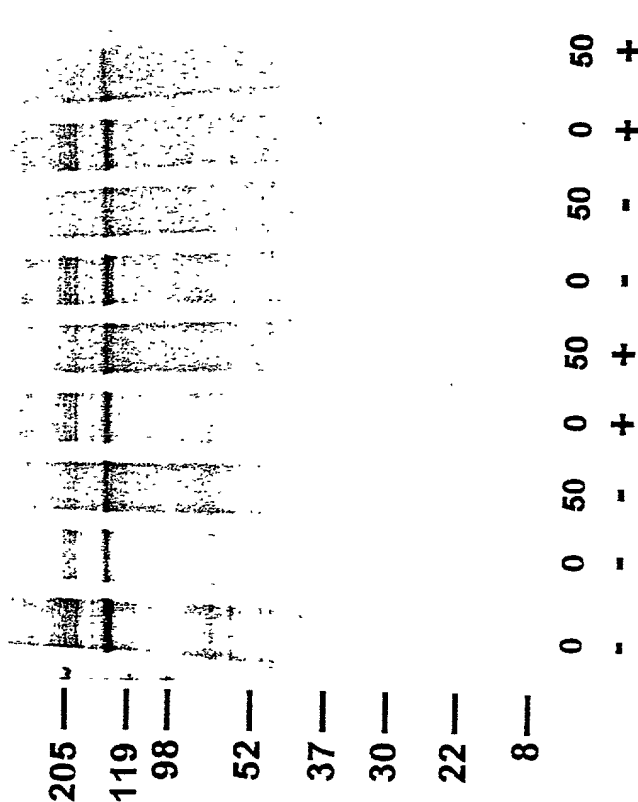
Regulated Freeze: decrease in temp. of 1°C/min to -56-59°C, then placed in -80°C

7A

# Guanidine Extract of Porcine ACL Irradiated in Cryopreservative ± Ascorbate at -80°C at 5.1 kGy/h

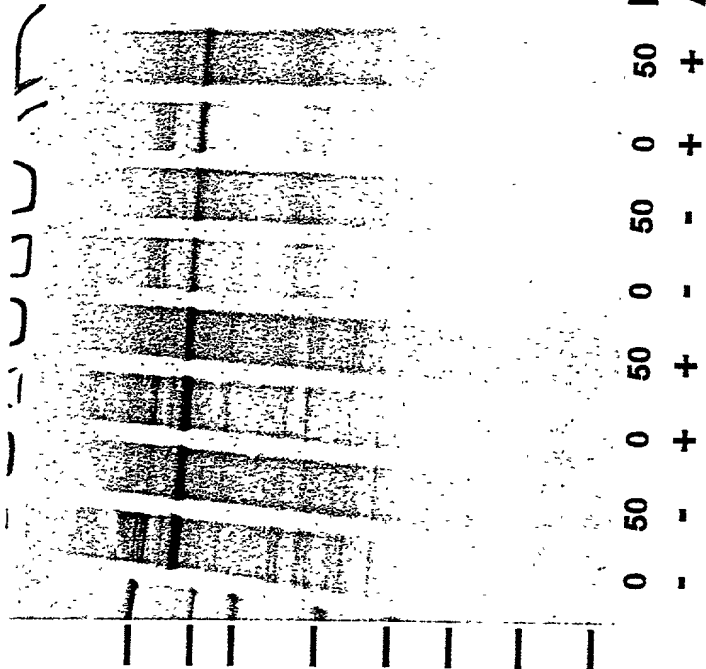
## Edmonton Cryopreservative

FC Quick Regulated



## Modified VS55

Quick Regulated



Edmonton CP: M199, 10% FCS, 2 M DMSO, Pen-Strep

Modified VS55: 100 mM trehalose, 15 mM  $\text{KH}_2\text{PO}_4$ , 42 mM  $\text{K}_2\text{HPO}_4$ , 150 mM mannitol, 24.2% DMSO, 16.8% 1,2-propanediol, 14% formamide

FC: fresh ACL control

Quick Freeze: dry-ice ethanol bath

Regulated Freeze: decrease in temp. of 1°C/min to -56-59°C, then placed in -80°C

78

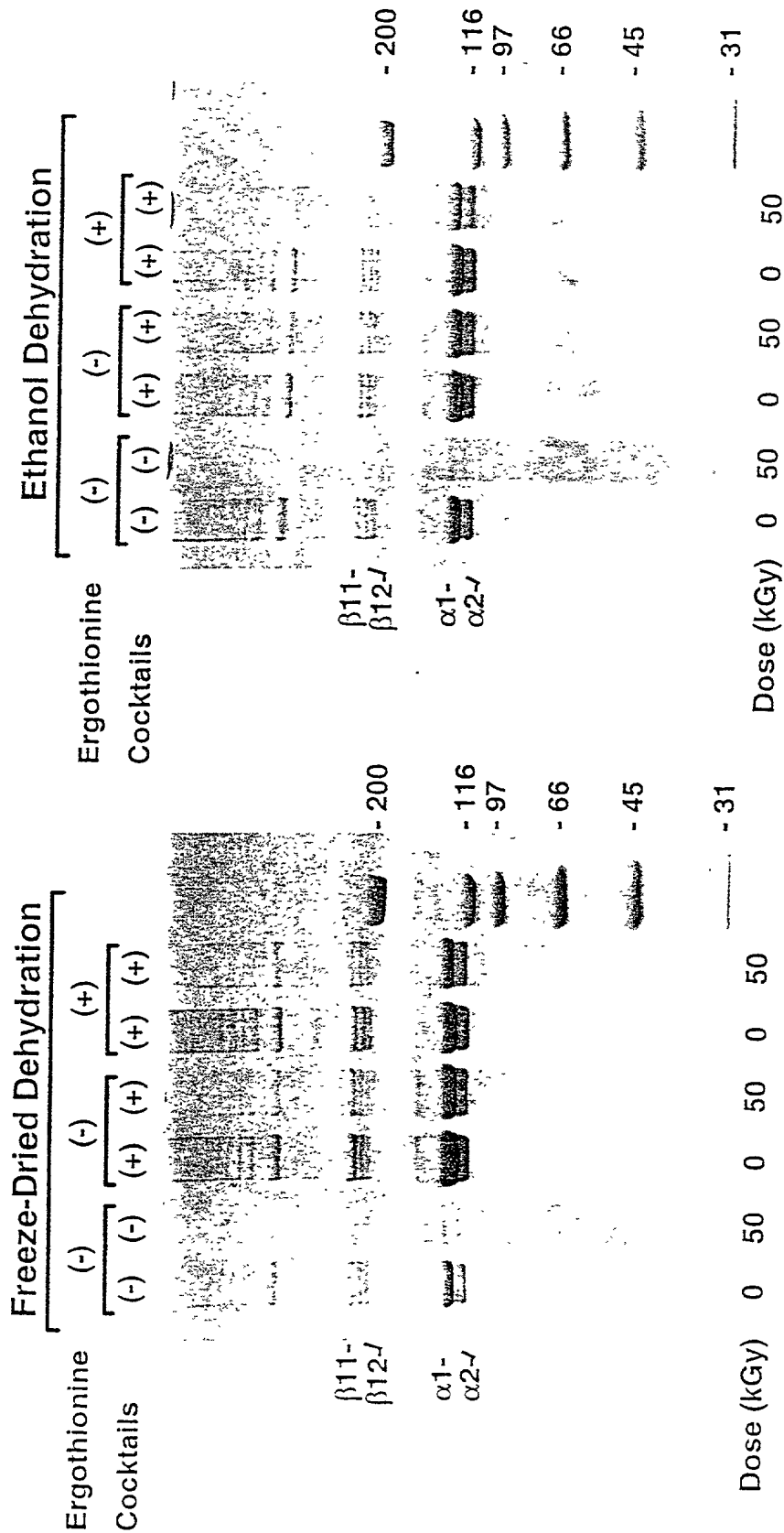
Regulated Freeze 1°C/min		Quick Freeze										
Ascorbate	VS55				CP				Dose (kGy)			
	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)				
-200									0	50	0	50
-116									0	50	0	50
-97									0	50	0	50
-66									0	50	0	50
-45									0	50	0	50
-31									0	50	0	50
-21									0	50	0	50

**VS55:** 100mM trehalose, 15mM  $\text{KH}_2\text{PO}_4$ , 42mM  $\text{K}_2\text{HPO}_4$ , 15mM KCl, 10mM  $\text{NaHCO}_3$ , 150mM mannitol, 24.2% DMSO, 16.8% 1,2-propanediol, and 14% formamide

CP: 10% FCS, Penicillin-streptomycin, M199, and 2M DMSO

U  
H

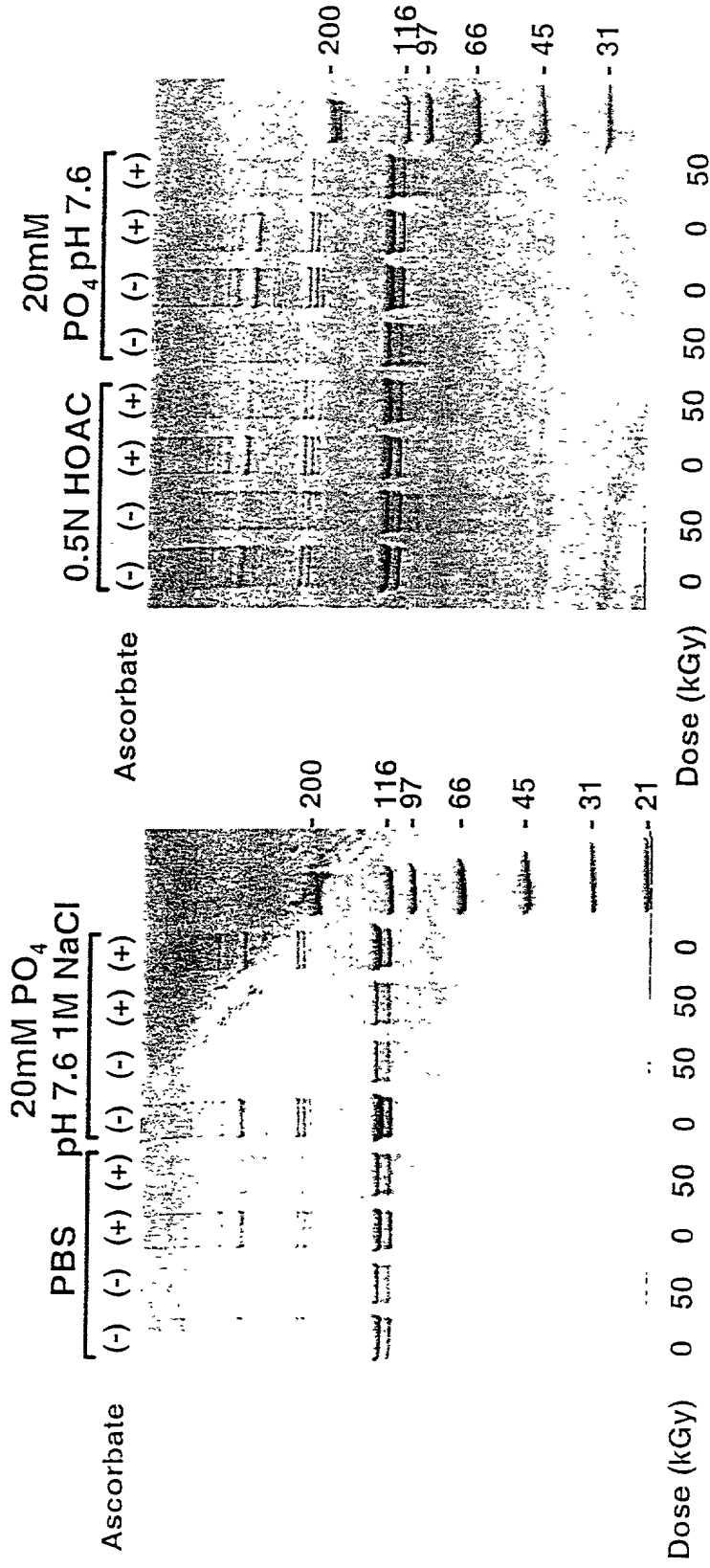
# Pepsin-Digested Collagen Isolated from Irradiated Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C 1.656kGy/hr



Cocktails: 100µM trolox, 100mM ascorbic acid, 100mM lipoic acid, 100mM n-propyl gallate

Fig. 8

Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Ascorbate at -80°C 1.53kGy/hr )



GA

[illegible]

Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Scavengers at -80°C 1.53kGy/hr

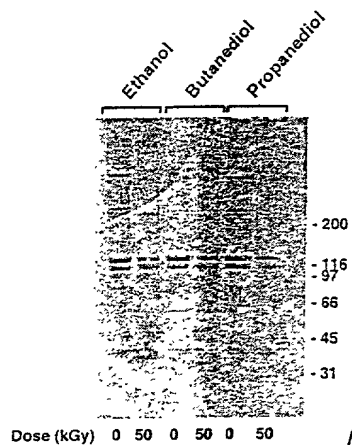
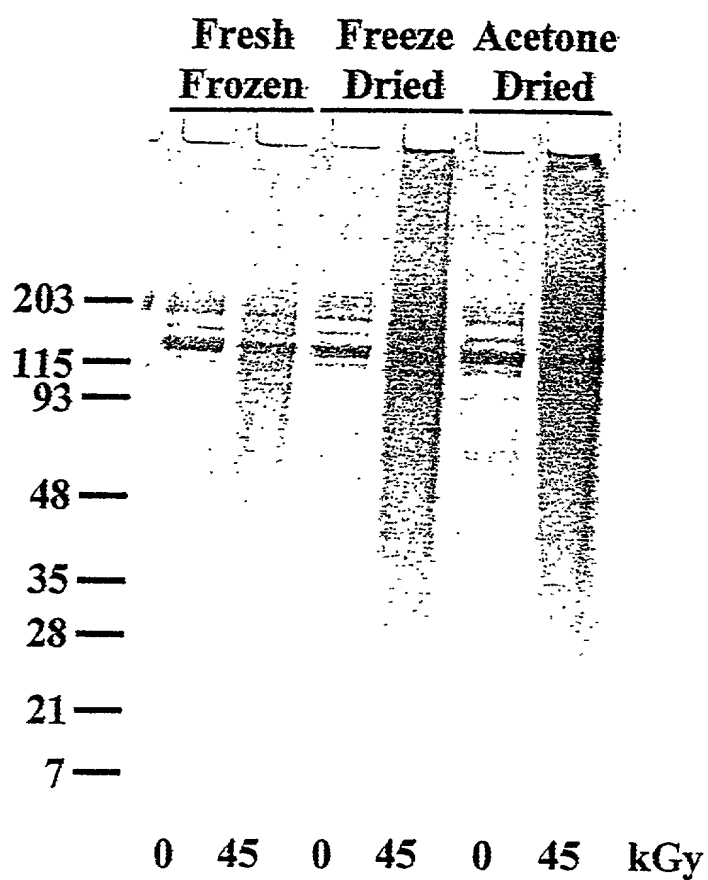


fig. 10



# Low Temperature Gamma Irradiation of ACLs Subjected to Various Forms of Preservation -80°C, 1.5 kGy/h



//

**$\gamma$ -irradiation of type I Freeze-Dried Collagen in the Presence of Antioxidants  
at 4°C 1.656kGy/hr I**

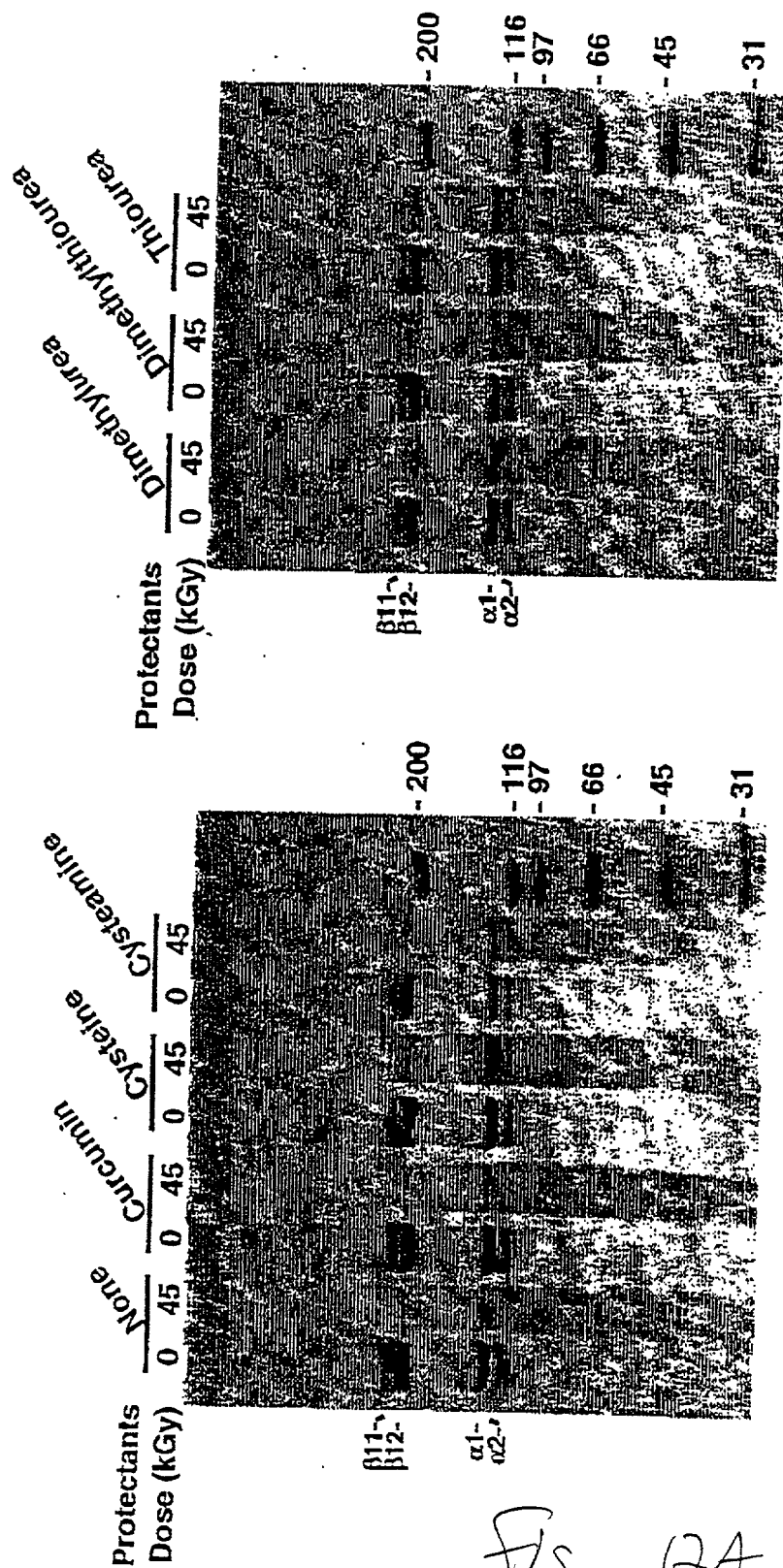


Fig. 12A

**$\gamma$ -irradiation of type I Collagen Solution and Gel in the Presence of Antioxidants  
at -20°C 1.537kGy/hr**

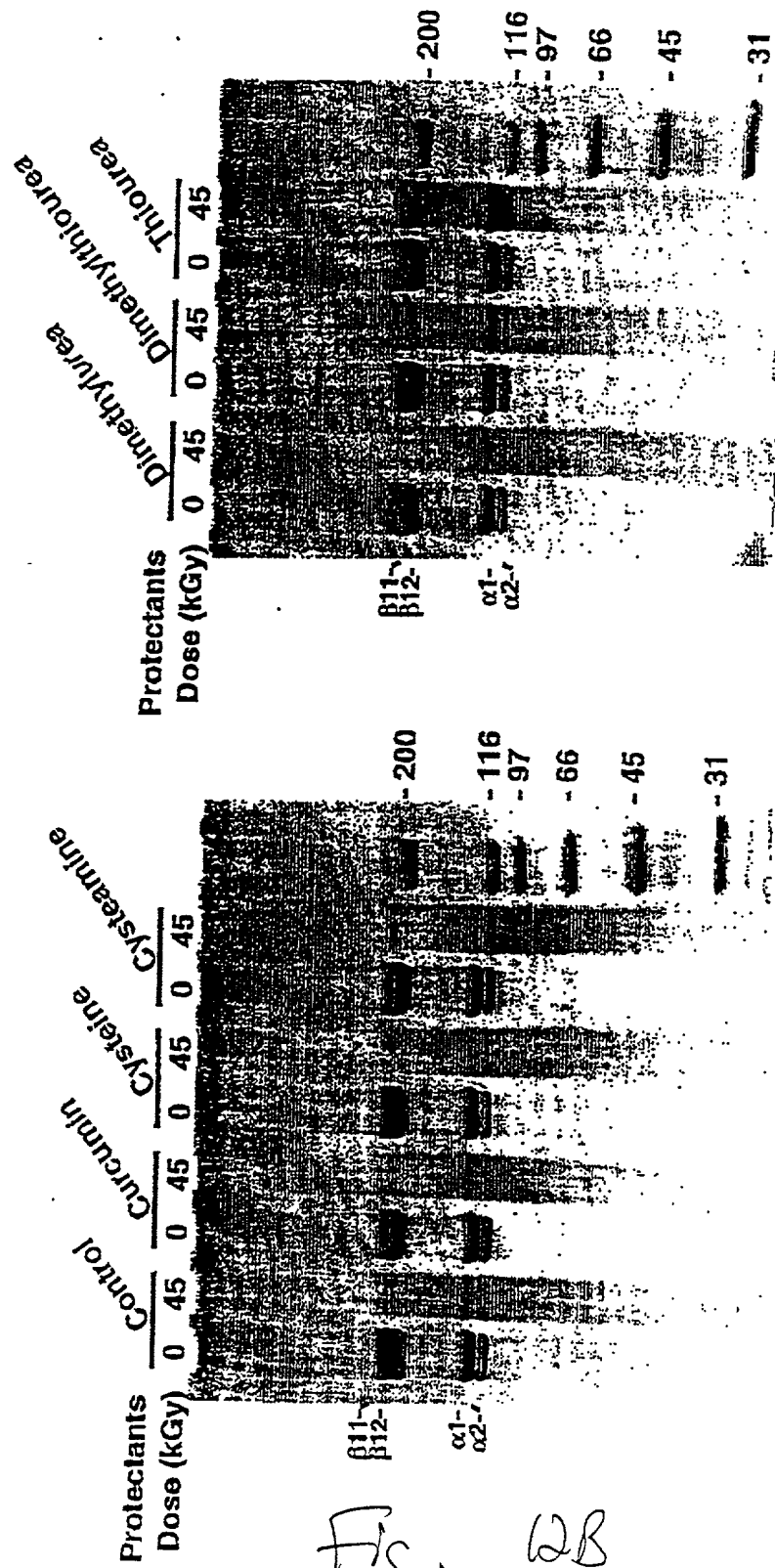


Fig. 2B

**$\gamma$ -irradiation of type I Collagen Solution In the Presence of Antioxidants  
at  $-80^{\circ}\text{C}$  1.53kGy/hr**

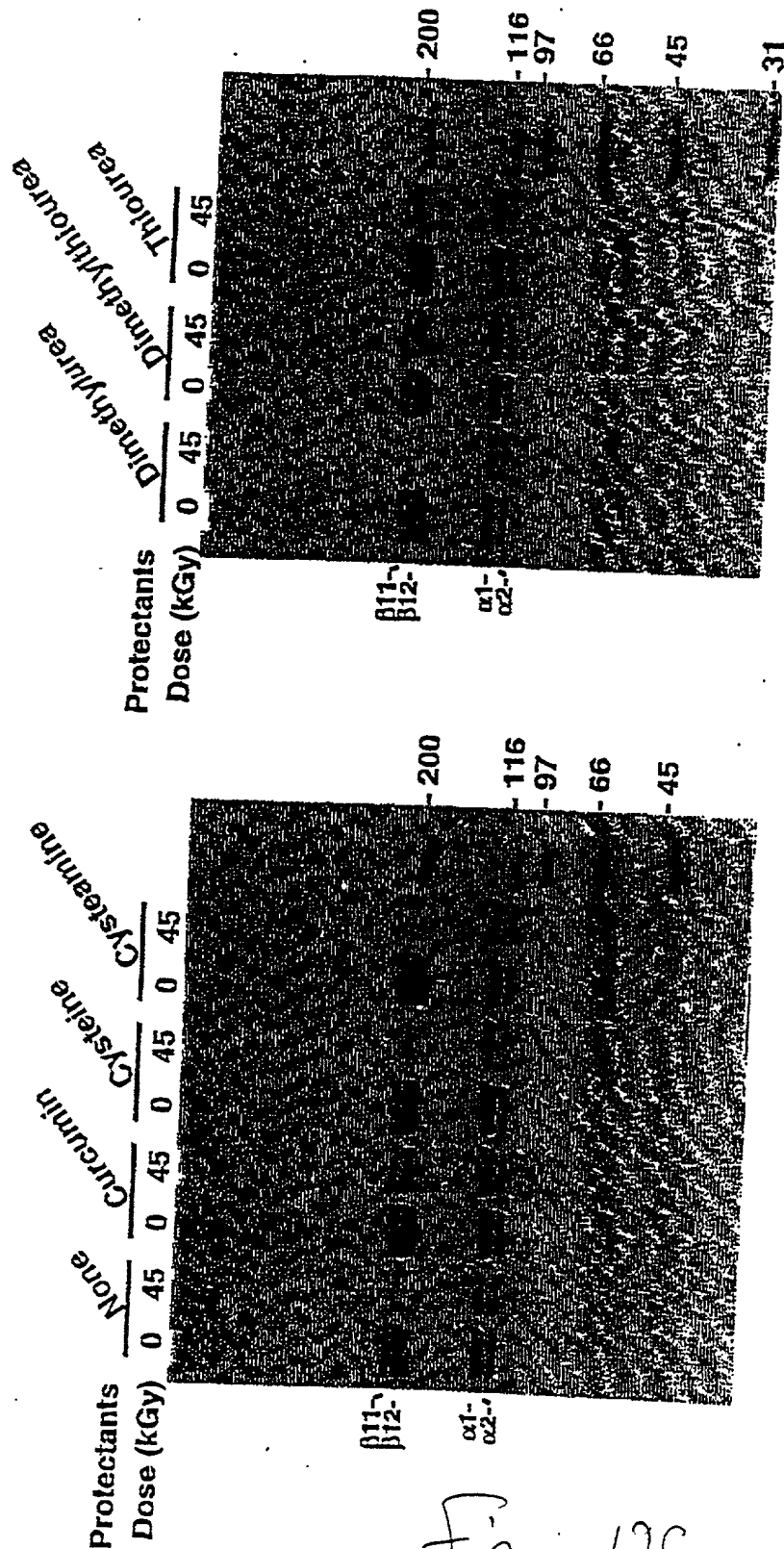


Fig. 12C

**$\gamma$ -irradiation of type I Collagen Solution and Gel in the Presence of Antioxidants  
at -80°C 1.3kGy/hr**

Protectants	Control			Asc			Asc/GG			Protectants			Asc/GG Methionine			Thiourea		
	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose

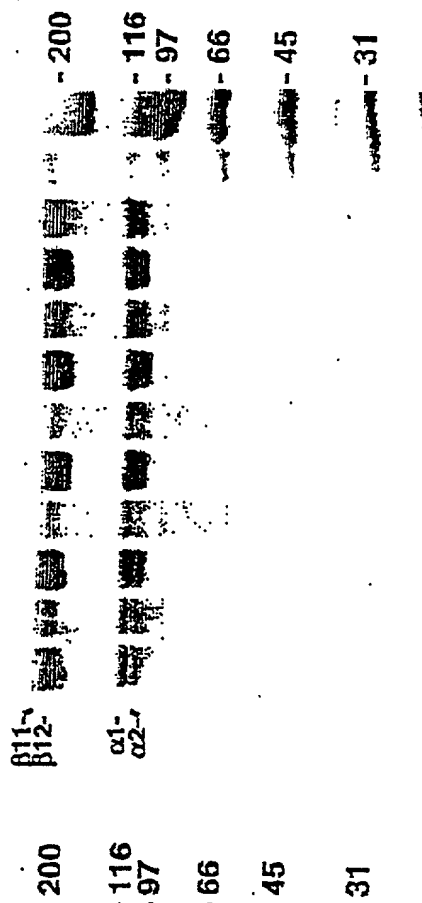
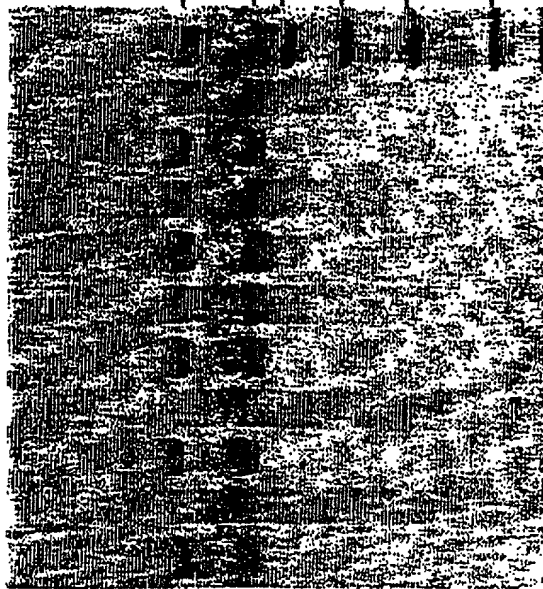


Fig. 13

$\gamma$ -irradiation of Freeze-Dried type I Collagen in the Presence of Antioxidants at 4°C 1.673kGy/hr

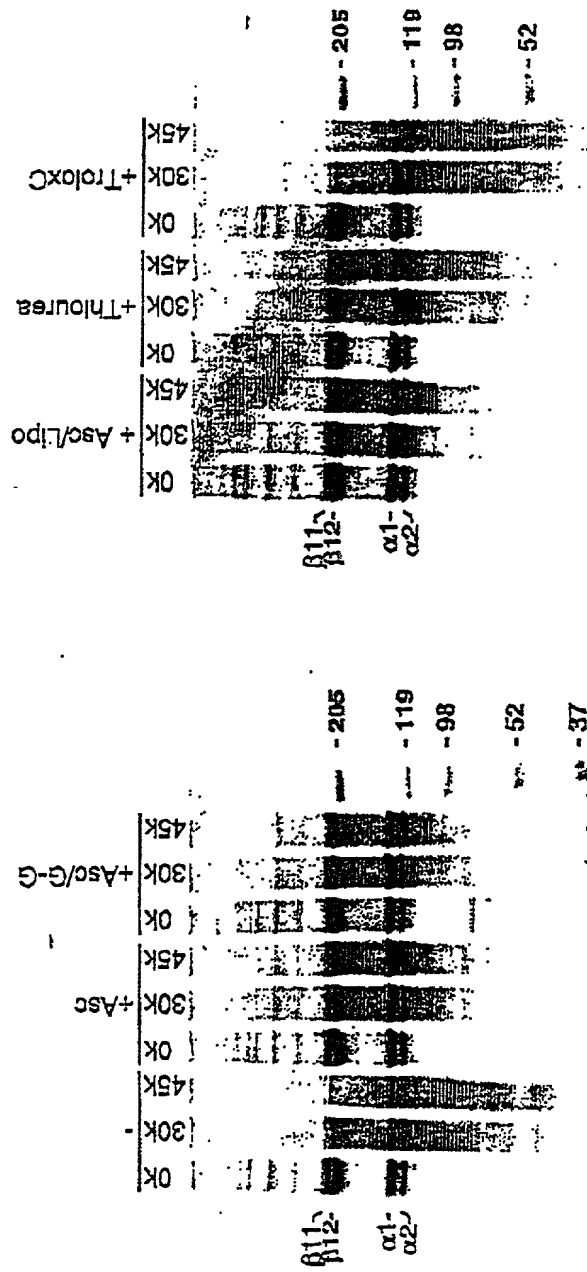


Fig. 14A

$\gamma$ -irradiation of type I Collagen Solution in the Presence of Antioxidants at 4°C kGy/hr

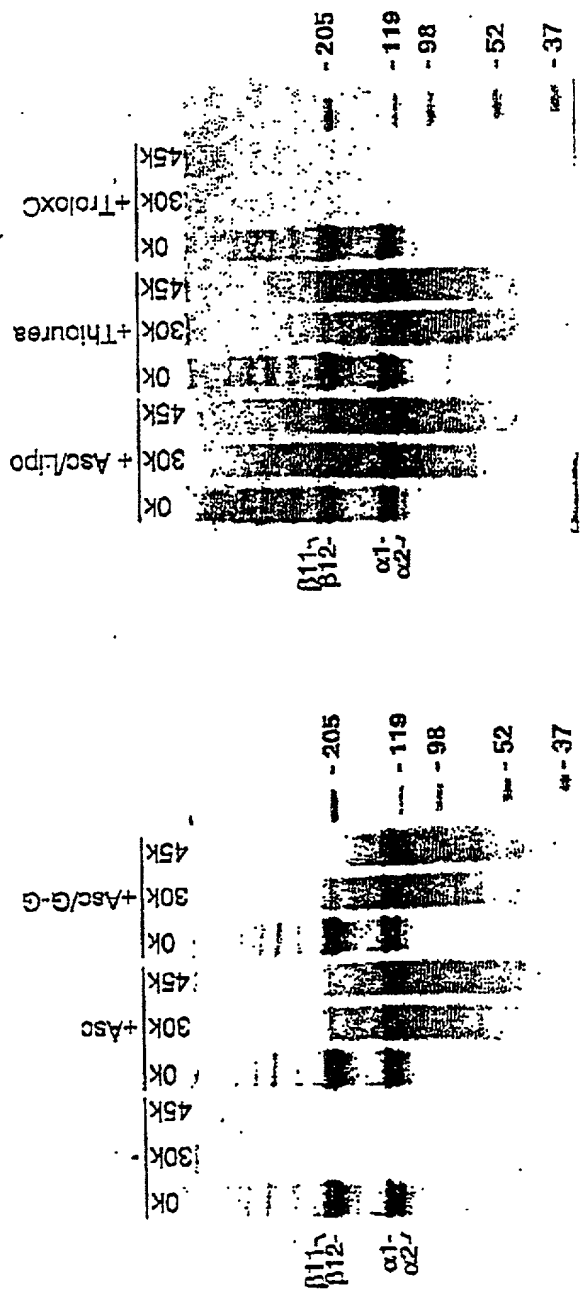


Fig. 14B

$\gamma$ -irradiation of type I Collagen Solution in the Presence of Antioxidants at -20°C 1.552kGy/hr

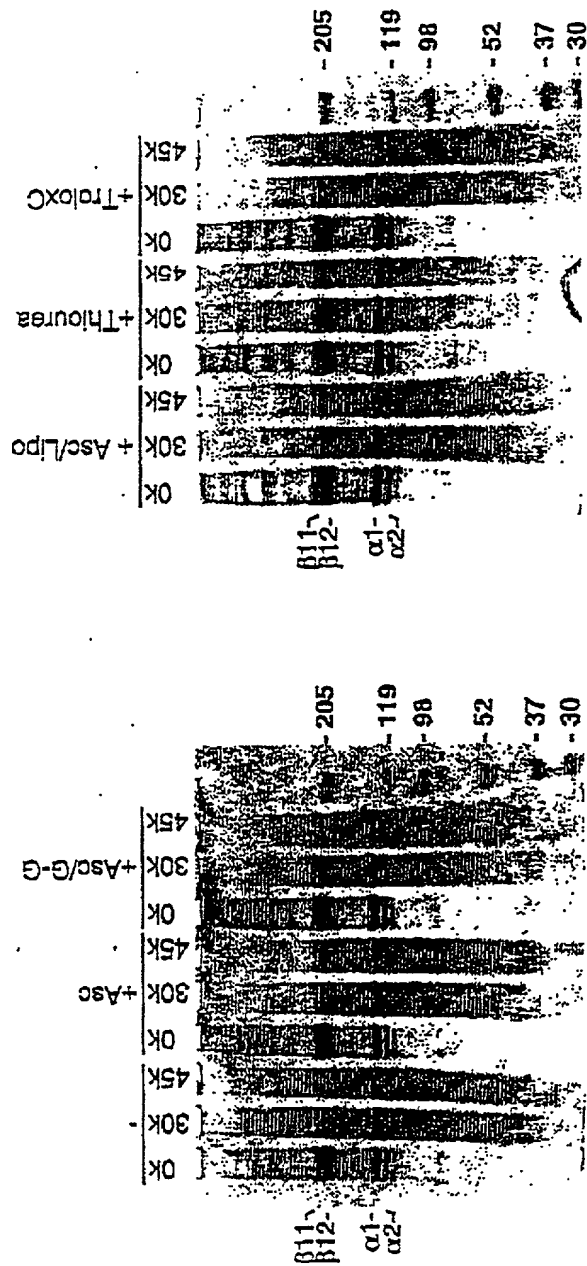


Fig. 14C



$\gamma$ -irradiation of type I Collagen Solution in the Presence of Antioxidants at  $-80^{\circ}\text{C}$  5.136kGy/hr



Figure 14D